

# *N*-Acetyl-L-Cysteine Enhances Interleukin-1 $\beta$ -Induced Nitric Oxide Synthase Expression

Bingbing Jiang, Michael Haverty, Peter Brecher

**Abstract**—The effect of *N*-acetyl-L-cysteine on interleukin-1 $\beta$ -induced nitric oxide synthase expression was studied in rat vascular smooth muscle cells to determine if the reduction/oxidation state would modulate cytokine-induced changes. Interleukin-1 $\beta$  induced the production of nitrite, a stable metabolite of nitric oxide in a time- and dose-dependent manner. Cytokine-induced nitrite production was enhanced by the addition of *N*-acetyl-L-cysteine in a dose-dependent manner, with a >50% increase produced by the addition of 1 mmol/L *N*-acetyl-L-cysteine. There was no influence on nitrite production when the cells were treated with *N*-acetyl-L-cysteine alone. Northern and Western blot analyses revealed that the upregulation of interleukin-1 $\beta$ -induced nitric oxide production by *N*-acetyl-L-cysteine resulted from an enhanced expression of inducible nitric oxide synthase. Interferon- $\gamma$  or tumor necrosis factor- $\alpha$  when used alone had no influence on nitrite production in the absence or presence of *N*-acetyl-L-cysteine. Nitrite accumulation was higher by the cells treated with interleukin-1 $\beta$  combined with either interferon- $\gamma$  or tumor necrosis factor- $\alpha$  compared with those treated with interleukin-1 $\beta$  alone. *N*-Acetyl-L-cysteine upregulated nitrite production and inducible nitric oxide synthase expression induced by combination treatment with interleukin-1 $\beta$  and either interferon- $\gamma$  or tumor necrosis factor- $\alpha$ . However, *N*-acetyl-L-cysteine had no significant influence in cytokine-induced activation of nuclear factor- $\kappa$ B or signal transducer and activator of transcription-1, as assessed by electrophoretic mobility shift assays. These results demonstrate that *N*-acetyl-L-cysteine possibly acted as a thiol-containing reducing agent and facilitated the expression of inducible nitric oxide synthase in rat vascular smooth muscle cells by cytokines through a mechanism that is independent of nuclear factor- $\kappa$ B or signal transducer and activator of transcription-1. (*Hypertension*. 1999;34:574-579.)

**Key Words:** acetylcysteine ■ cytokines ■ nitric oxide ■ RNA ■ interleukins ■ muscle, smooth, vascular

Nitric oxide (NO) is a multifunctional molecule that mediates many physiological and pathophysiological processes.<sup>1,2</sup> The expression of the inducible isoform of NO synthase (iNOS), which subsequently results in a large amount of NO production, has been demonstrated in neointimal smooth muscle cells of balloon-injured arteries of experimental animals<sup>3-5</sup> and in human atherosclerotic lesions.<sup>6,7</sup> Immunostaining and in situ hybridization confirmed the presence of iNOS in human atherosclerotic vessels, in which it was specifically localized to macrophages, foam cells, and vascular smooth muscle cells (VSMCs).<sup>6,7</sup> Although the specific functions of the NO produced by iNOS in injured vessels remain speculative, evidence from several studies indicate that NO has an important role in the prevention of leukocyte and platelet adhesion,<sup>4,5</sup> the modulation of vascular tone,<sup>4,8</sup> and the inhibition of smooth muscle cell and fibroblast proliferation.<sup>9-11</sup> In addition, delayed wound healing has been observed in iNOS knockout mice and this delayed wound healing could be completely reversed by application of an adenovirus vector that contained human iNOS cDNA at the time of injury.<sup>12</sup> These observations suggest an important clinical significance of NO synthesis

induced by cytokines in atherogenesis or vascular remodeling.

By altering the reduction/oxidation state of the cell, oxidative stress is thought to influence cellular responses during inflammation and is apparently involved in the pathogenesis of vascular diseases such as hypertension and atherosclerosis.<sup>13-15</sup> Antioxidants have been shown to slow the atherogenic process in several experimental models.<sup>14</sup> Evidence from human studies also suggests a beneficial effect of antioxidants in the prevention of atherosclerosis, although the overall roles of antioxidants require further elucidation.<sup>15</sup> The expression of iNOS represents an example of cellular responses to stimulation of proinflammatory cytokines. However, whether and how the reduction/oxidation state affects iNOS expression in vascular cells under cytokine stimulation are unresolved questions. In the present study, we used *N*-acetyl-L-cysteine (NAC), a widely used thiol-containing antioxidant that is able to support intracellular glutathione synthesis and scavenge reactive oxygen intermediates, to examine how it might influence the cytokine induction of NO production and iNOS expression in cultured VSMCs from rat thoracic aorta.

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## Methods

### Materials

DMEM/Ham's F12 medium (DMEM/F12), FCS, and a penicillin/streptomycin/amphotericin B mixture were purchased from Life Technologies, Inc. Recombinant human interleukin-1 $\beta$  (IL-1 $\beta$ ) (specific activity:  $\approx 1 \times 10^8$  U/mg) was purchased from Genzyme Corp. Recombinant rat interferon- $\gamma$  (IFN- $\gamma$ ) (specific activity:  $\approx 1 \times 10^7$  U/mg) and rat tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (specific activity:  $\approx 2 \times 10^7$  U/mg) were purchased from Pepro Tech, Inc. NAC was obtained from Sigma Chemical Co. NADPH, FAD, and nitrate reductase were obtained from Boehringer Mannheim. Rabbit anti-mouse macrophage iNOS (1131-1144) was obtained from Calbiochem. Horseradish peroxidase-linked anti-rabbit antibody was obtained from New England BioLabs. [ $\alpha$ - $^{32}$ P]dCTP (111 TBq/mmol) and [ $\gamma$ - $^{32}$ P]ATP (111 TBq/mmol) were purchased from DuPont-New England Nuclear. All other materials used were commercial products of the highest grade available.

### Cell Culture

VSMCs were isolated from rat thoracic aorta of 8-week-old male Wistar rats by an explant method<sup>16</sup> and cultured in DMEM/F12 that was supplemented with 10% FCS, 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin, and 0.25 mg/L amphotericin B at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub>. The cells were used between passages 4 and 6.

### Determination of Nitrite

Cells that were released with 0.05% trypsin/0.02% EDTA solution (GIBCO) from stock cultures seeded on 24-well culture plates in DMEM/F12 with 10% FCS. At confluence after 3- or 4-day incubation, the cells were washed twice with and then cultured in phenol red-free DMEM/F12 that contained 0.1% FCS for 24 to 48 hours. The medium was changed, and various agents were added as indicated in Results. After the designated time, the release of NO from cultures was assessed by the determination of nitrite, a stable metabolite of NO in conditioned medium, with Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine in 2% phosphoric acid). The absorbance at 570 nm was read by a microplate reader (Dynatech MR 600). The nitrite concentrations of samples were calculated with a series of known concentrations of sodium nitrite as a standard and expressed as  $\mu$ mol/L. To examine whether NAC might modulate IL-1 $\beta$ -induced nitrite production by altering the ratio of nitrite and nitrate, total nitrite/nitrate levels were determined in some experiments by the use of nitrate reductase to convert nitrate to nitrite. Briefly, 50  $\mu$ L of conditioned medium was mixed with 50  $\mu$ L of distilled water or 50  $\mu$ L of freshly prepared reaction solution that contained 0.2 U/mL nitrate reductase, 0.2 mmol/L NADPH, and 0.02 mmol/L FAD. After incubation for 1.5 hours at room temperature, 100  $\mu$ L of Griess reagent was added and the absorbance at 570 nm was read. Nitrate concentration was obtained by subtraction of the nitrite concentration (without conversion reaction) from the total nitrite/nitrate concentration (with conversion reaction).

### Northern Blot Analysis

VSMCs cultured in 100-mm Petri dishes at confluence were kept in DMEM/F12 that contained 0.1% FCS for 24 to 48 hours and then incubated in the same but fresh medium with or without other agents for 16 hours. Total RNA was then extracted from the cells as described previously<sup>17</sup> with Trizol reagent (GIBCO-BRL) instead of Isogen reagent. The concentration of RNA was estimated from the absorbance at 260 nm. The RNA (10  $\mu$ g per lane) was electrophoresed on 1% agarose-formaldehyde gels, transferred to nylon transfer membranes (Micron Separations, Inc), and cross-linked to membranes by UV irradiation. After prehybridization, the membranes were hybridized with randomly primed [ $\alpha$ - $^{32}$ P]dCTP-labeled cDNA probe for iNOS (an insert of *Kpn*I-*Bam*HI restriction fragment of the rat iNOS plasmid<sup>18</sup>) at 65°C for 16 hours. The membranes were washed in a serial dilution of solutions starting at 1 $\times$  SSC/0.5% SDS

and ending at 0.25 $\times$  SSC/0.125% SDS at 65°C (1 $\times$  SSC contains 150 mmol/L NaCl and 15 mmol/L sodium citrate, pH 7.0). Autoradiography was performed by exposure of the membranes to Hyperfilm (Amersham) at -80°C with intensifying screens. As a reference, the blot was stripped off and reprobated with [ $\alpha$ - $^{32}$ P]dCTP-labeled cDNA probe for rat  $\beta$ -actin.<sup>18</sup>

### Western Blot Analysis

For detection of iNOS protein, confluent VSMCs cultured in 100-mm Petri dishes were maintained in DMEM/F12 that contained 0.1% FCS for 24 to 48 hours and incubated in fresh DMEM/F12 that contained 0.1% FCS, with or without other agents as indicated in Results. After a 24-hour incubation, the cells were washed once with ice-cold phosphate buffered saline and scraped into 0.5 mL of ice-cold cell lysis buffer that contained 20 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L  $\beta$ -glycerolphosphate, 1 mmol/L Na<sub>3</sub>VO<sub>4</sub>, 1  $\mu$ g/mL leupeptin, and 1  $\mu$ g/mL aprotinin. After incubation at 4°C for 30 minutes, the lysates were centrifuged at 10 000g for 15 minutes. Protein content of the lysate supernatants was determined with BCA protein assay reagent (Pierce), with bovine serum albumin used as a standard. Protein samples (30  $\mu$ g) from each lysate were separated on 10% SDS-polyacrylamide gels (2.6% C) and then electrotransferred to nitrocellulose membranes (BioRad). Membranes were blocked for 1 hour at room temperature in a buffer that contained 20 mmol/L Tris, pH 7.6, 137 mmol/L NaCl, 0.1% Tween 20, and 5% nonfat dry milk, and then incubated at 4°C overnight with 1:3000 diluted rabbit anti-mouse macrophage iNOS in blocking buffer. After the membranes were washed, they were incubated for 1 hour at room temperature with 1:2000 diluted HRP-linked anti-rabbit antibody and visualized with a chemiluminescence-based detection system (ECL, Amersham).

### Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared by the method of Schreiber et al<sup>19</sup> from cells cultured in 100-mm Petri dishes at the designated time after treatment with or without stimuli. Five micrograms of nuclear proteins were used for electrophoretic mobility shift assay (EMSA). Both double-stranded oligonucleotides that contained nuclear factor- $\kappa$ B (NF- $\kappa$ B) consensus sequence (5'-AGT TGA GGG GAC TTT CCC AGG C-3'; Promega) and *sis*-inducible element (SIE, 5'-GTG CAT TTC CCG TAA ATC TTG TCT ACA-3'; Santa Cruz) were end-labeled with [ $\gamma$ - $^{32}$ P]ATP. The binding reaction was performed for 20 minutes at room temperature in a total volume of 25  $\mu$ L of binding buffer that contained 22.5  $\mu$ g/mL poly(dI-dC), 20 mmol/L HEPES, pH 7.9, 1 mmol/L EDTA, 5 mmol/L MgCl<sub>2</sub>, 10% glycerol, 1 mmol/L PMSF, 1 mmol/L dithiothreitol, and salt (final concentration, 55 mmol/L KCl for NF- $\kappa$ B and 65 mmol/L NaCl for SIE). DNA-protein complexes were resolved on 4% nondenaturing polyacrylamide gel (5% C). The electrophoresis was for 2 hours 30 minutes at 200 volts in 0.5 $\times$  TBE (1 $\times$  TBE=90 mmol/L Tris-Borate, pH 8.0, 2 mmol/L EDTA). The gels were dried and then exposed to autoradiography (Hyperfilm MP, Amersham) at -80°C for 1 to 3 hours.

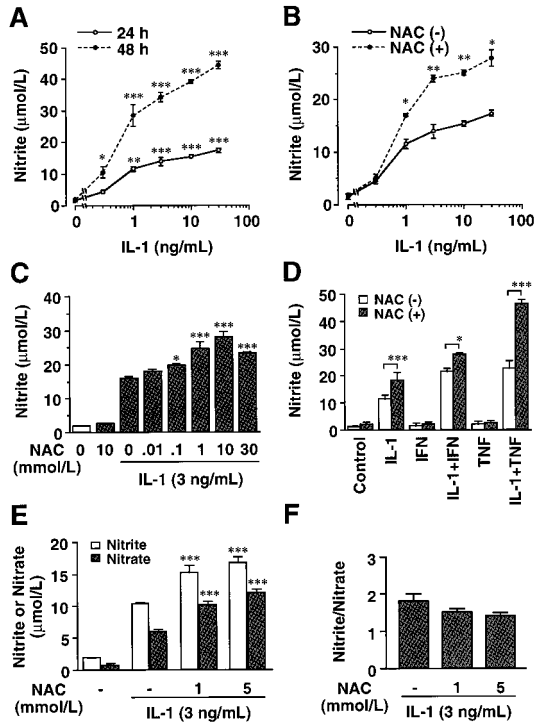
### Statistical Analysis

The nitrite data are expressed as mean  $\pm$  SD. Statistical analysis was performed by 1-way ANOVA and Student's *t* test with unpaired data with StatView version 4.01 (Abacus Concepts, Inc). *P* < 0.05 was considered to be significant.

## Results

### NAC Enhances IL-1 $\beta$ -Induced NO Production

IL-1 $\beta$  increased the production of nitrite, a stable metabolite of NO, by cultured rat VSMCs in a dose-dependent and time-dependent manner (Figure 1A). The IL-1 $\beta$ -induced nitrite production was significantly enhanced by the addition

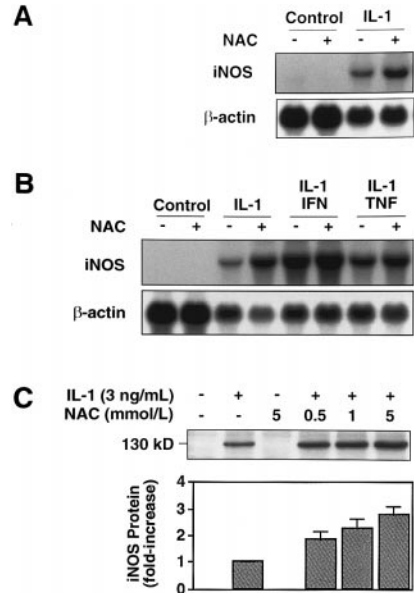


**Figure 1.** NAC enhances IL-1 $\beta$ -induced NO production in rat VSMCs. Cells were treated as follows: A, various concentrations of IL-1 $\beta$  for 24 and 48 hours; B, various concentrations of IL-1 $\beta$  with or without 1 mmol/L NAC for 24 hours; C, 3 ng/mL IL-1 $\beta$  with various concentrations of NAC for 24 hours; D, control, 3 ng/mL IL-1 $\beta$ , 200 U/mL IFN- $\gamma$ , 3 ng/mL IL-1 $\beta$  plus 200 U/mL IFN- $\gamma$ , 1000 U/mL TNF- $\alpha$ , and 3 ng/mL IL-1 $\beta$  plus 1000 U/mL TNF- $\alpha$ , with or without 1 mmol/L NAC for 24 hours; and E and F, control, 3 ng/mL IL-1 $\beta$  in the absence or presence of 1 or 5 mmol/L NAC for 24 hours. Values are mean  $\pm$  SD.  $n=2$  for A and B,  $n=3$  for C and D, and  $n=4$  for E and F. The graphs represent typical results from 3 or 4 individual experiments. \* $P<0.05$ , \*\* $P<0.01$ , and \*\*\* $P<0.001$ , significantly different from control (no addition, A), or IL-1 $\beta$  alone (B, C, and E), or as indicated (D). No significant differences existed ( $P>0.05$ ) in nitrite/nitrate ratios (F).

of NAC (Figure 1B) and this effect was dose-dependent (Figure 1C), with a >50% increase produced by the addition of 1 mmol/L NAC. At a high concentration (30 mmol/L), NAC caused nuclear condensation and detachment, which could explain the slight decrease in nitrite accumulation compared with NAC at 10 mmol/L (Figure 1C). NAC added alone at concentrations between 1 to 10 mmol/L showed no effect on nitrite production over a 24-hour period.

There was no significant production of nitrite by cells treated with either IFN- $\gamma$  (200 U/mL) or TNF- $\alpha$  (1000 U/mL). However, both IFN- $\gamma$  and TNF- $\alpha$  markedly enhanced IL-1 $\beta$ -induced nitrite production (Figure 1D). Nitrite accumulation in the cultures treated with IL-1 $\beta$  combined with either IFN- $\gamma$  or TNF- $\alpha$  was significantly higher in the presence of 1 mmol/L NAC than those with only cytokines added. Thus, NAC enhanced nitrite accumulation when added to cells treated either with IL-1 $\beta$  alone or with any combination of cytokines that caused nitrite production.

NAC enhanced both nitrite and nitrate production induced by IL-1 $\beta$  in a similar manner (Figure 1E). NAC at 1 or

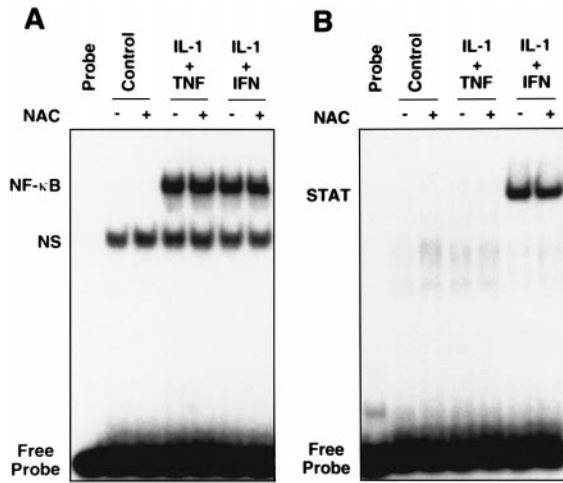


**Figure 2.** NAC enhances IL-1 $\beta$ -induced iNOS expression in rat VSMCs. A, Cells were incubated in medium without cytokines (lanes 1 and 2) or with 3 ng/mL IL-1 $\beta$  (lanes 3 and 4) in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 1 mmol/L NAC for 16 hours. B, Cells were incubated in medium without cytokines (lanes 1 and 2) or with 3 ng/mL IL-1 $\beta$  (lanes 3 and 4), 3 ng/mL IL-1 $\beta$  plus 200 U/mL IFN- $\gamma$  (lanes 5 and 6) or 1000 U/mL TNF- $\alpha$  (lanes 7 and 8) in the absence (lanes 1, 3, 5, and 7) or presence (lanes 2, 4, 6, and 8) of 1 mmol/L NAC for 16 hours. Total RNA was extracted and Northern blot analysis was performed with 10  $\mu$ g of total RNA per lane. The result shown in B represents 2 individual experiments. C, Cells were incubated in medium without any addition (lane 1) or with 3 ng/mL IL-1 $\beta$  (lane 2), 5 mmol/L NAC (lane 3), or 3 ng/mL IL-1 $\beta$  plus 0.5, 1, or 5 mmol/L NAC (lanes 4 to 6) for 24 hours. Cell lysates were prepared and 30  $\mu$ g of protein from each lysate was used in Western blot analysis. A rabbit anti-mouse macrophage iNOS was used and the 130-kDa iNOS protein was indicated. The result shown in C is representative of 3 separate experiments. The bar graph represents densitometric analysis of the immunoblot from 3 experiments with the arbitrary units from IL-1 $\beta$ -treated cells 1-fold.

5 mmol/L did not significantly affect the ratio of nitrite and nitrate (Figure 1F), suggesting no effect of NAC on the rates of NO breakdown to the stable end products of nitrite and nitrate.

### NAC Enhances IL-1 $\beta$ -Induced iNOS Expression

We further assessed whether the effect of NAC on nitrite production resulted from an increase in iNOS expression. Northern blot analysis showed that iNOS mRNA was obviously induced by treatment for 16 hours with IL-1 $\beta$  (Figure 2A), and this induction was enhanced by a combination of either IFN- $\gamma$  or TNF- $\alpha$  (Figure 2B). The steady state iNOS mRNA levels were higher in the presence of NAC than those in the absence of NAC. The iNOS mRNA was not detectable in untreated cells or the cells treated with NAC alone. Steady state mRNA levels for  $\beta$ -actin were downregulated by cytokine treatment but not affected by the addition of NAC (Figure 2A and 2B). Loading of the RNA from different samples was equivalent on the basis of visualization of the 28s and 18s ribosomal RNA.



**Figure 3.** NAC does not affect the activation of NF- $\kappa$ B and STAT-1 in rat VSMCs. Cells were not exposed to any cytokine (control) or they were exposed to 3 ng/mL IL-1 $\beta$  plus 1000 U/mL TNF- $\alpha$  or 3 ng/mL IL-1 $\beta$  plus 200 U/mL IFN- $\gamma$ , with or without 1 mmol/L NAC for 1 hour. Nuclear extracts were assayed for NF- $\kappa$ B (A) and STAT-1 (B) by EMSA. Lane 1 indicates reaction without nuclear extract; NS, non-specific binding.

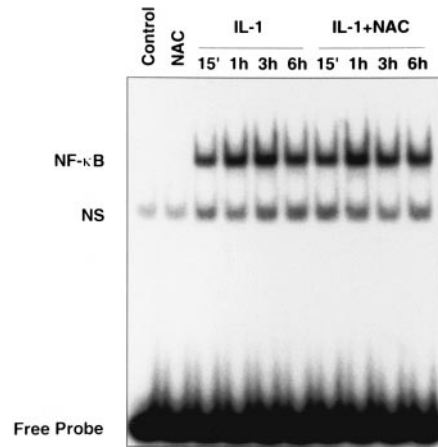
Furthermore, Western blot analysis was performed with an iNOS-specific polyclonal antibody. IL-1 $\beta$  treatment for 24 hours clearly induced the 130-kD iNOS protein expression as shown in Figure 2C. The iNOS protein was not detectable in untreated VSMCs. NAC added without cytokine at a concentration of 5 mmol/L did not induce iNOS, but clearly potentiated IL-1 $\beta$ -induced iNOS protein expression in a dose-dependent manner.

### NAC Has No Influence on NF- $\kappa$ B or STAT-1 Activation

To assess whether the effect of NAC on cytokine-induced expression of iNOS was secondary to an increase in activation of transcription factors known to be involved in iNOS expression, we used EMSA to determine if NAC would influence the nuclear translocation of NF- $\kappa$ B or signal transducer and activator of transcription (STAT)-1. NF- $\kappa$ B activation and translocation clearly occurred in response to IL-1 $\beta$  or TNF- $\alpha$  (Figure 3A) but was unaffected by the addition of IFN- $\gamma$  (data not shown). By contrast, IFN- $\gamma$  clearly activated STAT-1, whereas IL-1 $\beta$  and TNF- $\alpha$  had no effect (Figure 3B). NF- $\kappa$ B and STAT-1 were not detectable in the nuclear extracts treated with NAC alone and when NAC was added in combination with any of the cytokines, there was no obvious influence on the translocation of both NF- $\kappa$ B and STAT-1 (Figure 3A and B). Time-course study showed that NAC did not cause obvious alteration in IL-1 $\beta$ -induced activation of NF- $\kappa$ B between 15 minutes and 6 hours (Figure 4).

### Discussion

In many cell types, such as cardiac fibroblasts, iNOS expression is induced only when  $\geq 2$  cytokines are added, a finding explained in part by the necessity for activation of a complex of transcription factors that cannot be



**Figure 4.** NAC has no obvious influence in IL-1 $\beta$ -induced NF- $\kappa$ B activation. Cells were incubated in the medium without any addition (control), with 1 mmol/L NAC for 1 hour, or with 3 ng/mL IL-1 $\beta$  in the absence or presence of 1 mmol/L NAC for the time indicated. Nuclear extracts were assayed for NF- $\kappa$ B by EMSA.

activated by a single cytokine.<sup>18,20</sup> However, VSMCs have been shown to respond to IL-1 $\beta$  alone with increased iNOS expression.<sup>17,21</sup> This response to IL-1 $\beta$  was confirmed in this study and used as a model system to examine how NAC, a substance often used to examine the role of oxidative stress in a variety of cell types, might influence the induction of iNOS in VSMCs.

The present study clearly demonstrated that NAC, a thiol-containing antioxidant, enhanced IL-1 $\beta$ -induced nitrite production, and this effect was apparently associated with the increased iNOS mRNA transcription and protein expression. Neither IFN- $\gamma$  nor TNF- $\alpha$  alone could induce nitrite production at tested concentrations. However, IFN- $\gamma$  or TNF- $\alpha$  could enhance IL-1 $\beta$ -induced nitrite production and iNOS expression, which suggests a complementary effect of the signal transduction by combination of the cytokines. The effect of NAC on cytokine induction of NO was most evident when cells were treated with IL-1 $\beta$  and TNF- $\alpha$ , which implicated that selective signaling pathways of IL-1 $\beta$  and TNF- $\alpha$  were more sensitive to the antioxidant.

It is well known that NF- $\kappa$ B activation is essential for the induction of iNOS.<sup>22,23</sup> NAC has been reported to inhibit NF- $\kappa$ B activation or translocation in some cell types (macrophages, Kupffer cells, mesangial cells, and astrocytes),<sup>24,25</sup> which might be a possible mechanism involved in the inhibition of iNOS induction.<sup>24</sup> However, NAC failed to inhibit iNOS induction in rat hepatocytes.<sup>26</sup> In hepatocytes, intracellular glutathione was reported to be required for cytokine-induced NF- $\kappa$ B activation and iNOS expression because the iNOS induction was prevented by glutathione depletion and could be restored by addition of NAC.<sup>27,28</sup> The results from the present study showed no obvious effect of NAC on cytokine activation of NF- $\kappa$ B, which was consistent with previous reports<sup>29</sup> that indicated no inhibitory effect of NAC on IL-1- and TNF-activated NF- $\kappa$ B in some cell lines. Similarly, we showed no

influence of NAC in cytokine-activated STAT-1. It has been reported that DNA binding of NF- $\kappa$ B was only inhibited at concentrations of NAC >20 mmol/L and that low-dose NAC protected rats against endotoxin-mediated oxidative stress, but high-dose NAC increased mortality.<sup>30</sup> In our study, cell damage was observed in the cultures with a high concentration of NAC (30 mmol/L). The effective concentrations of NAC on potentiating IL-1 $\beta$  induction of iNOS were relatively lower than those usually used for inhibiting NF- $\kappa$ B activation. Because NAC may provide additional cysteine to the cultured cells, it has been suggested that the concentration of cysteine may be insufficient to ensure maximal NO synthase activity.<sup>28</sup> However, the addition of IFN- $\gamma$  or TNF- $\alpha$  significantly elevated IL-1 $\beta$ -induced nitrite production and iNOS expression in the same media without the addition of NAC or cysteine, thus it is unlikely that cysteine concentration in the culture media was a restrictive factor.

Although the expression of iNOS is associated with infection or inflammation and is geared toward host defense, the role of iNOS expressed in the vascular wall during atherosclerotic injury remains to be clarified. A variety of evidence indicate that NO plays an important antiatherogenic role by inhibiting VSMC proliferation, platelet aggregation, lipoprotein oxidation, and expression of adhesion molecules.<sup>1,4,9,10,31,32</sup> Long-term inhibition of NO synthesis has been shown to promote atherosclerosis,<sup>33,34</sup> whereas supplementation with L-arginine was reported to reduce atherogenesis.<sup>35</sup> However, if a large amount of NO reacts with superoxide anion to produce highly reactive peroxynitrite, the latter can directly modify lipids and proteins and is cytotoxic. NAC has been reported to be effective on hepatoprotection and pneumoprotection from inflammatory injury,<sup>36,37</sup> and reduce cerebral infarction during reperfusion after temporary middle cerebral artery occlusion.<sup>38</sup> The beneficial effects of NAC on protecting tissues from damage are most probably a result of its antioxidant properties and its influence in NO production. It is plausible to hypothesize that NAC could be useful for elevating the antiatherogenic effect of NO via the increase in cytokine-induced NO production and inhibition of peroxynitrite formation.

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