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Thioredoxin Inhibits Tumor Necrosis Factor- or Interleukin-1-Induced NF-κB Activation at a Level Upstream of NF-κB-Inducing Kinase

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ABSTRACT

Gene induction by tumor necrosis factor-α (TNFα) or interleukin-1β (IL-1β) is mediated in part by activation of the transcription factor nuclear factor κB (NF-κB), and requires signal adaptor molecules such as TNF receptor-associated factor (TRAFs). The latter interact with the NF-κB-inducing kinase (NIK), which is believed to be part of the IκB kinase complex. Although the precise mechanism is to be elucidated, it is well-known that antioxidant treatments inhibit the inflammatory cytokine-induced NF-κB activation. Thioredoxin (TRX) is a 12-kDa endogenous protein that regulates various cellular functions by modulating the redox state of proteins, overexpression of this molecule inhibits NF-κB activation. To elucidate the roles of TRX in the signal transduction of the cytokines, we investigated the effects of TRX on NF-κB activation induced by cytokine treatment or by overexpression of the signaling molecules. Our data show that TRX treatment inhibits NF-κB-dependent transcription at the level of downstream of TRAFs and upstream of NIK: TRX inhibited TRAF2-, TRAF5-, and TRAF6-induced NF-κB activation but does not inhibit NIK-, IKKα-, and MEKK-induced activation. In addition, we show that TRX inhibits NF-κB activation in a manner different from that for SAPK (stress activated protein kinase) inhibition. Antiox. Redox Signal. 2, 83–92.

INTRODUCTION

CELLS HAVE MULTIPLE PATHWAYS to transduce extracellular signals into the nuclear compartment. These pathways are complex networks that ultimately modulate gene expression. Intermediary proteins in the transmission of signals from the cell surface to the nucleus are numerous and incompletely understood. Kinases and phosphatases represent signal transducers that regulate activity by phosphorylation and dephosphorylation. Oxidants and antioxidants represent a different set of signaling molecules that modify function through redox (Pahl and Baeuerle, 1994; Sen and Packer, 1996; Suzuki *et al.*, 1997; Dalton *et al.*, 1999). Similar to phosphorylation, redox can serve as the critical switch in many processes. Biologically relevant oxidants: hydrogen peroxide (H_2O_2) and nitric oxide (NO), that serve as pleiotropic

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signaling molecules have been well documented (Meyer *et al.*, 1993; Sundaresan *et al.*, 1995; Irani *et al.*, 1997). Balancing these oxidants are antioxidants such as glutathione, glutaredoxin, and thioredoxin (TRX) (Tagaya *et al.*, 1989; Nakamura *et al.*, 1997). The delicate interplay inside cells between oxidants and antioxidants ultimately determines the activity profile for many transcription factors (Pahl and Baeuerle, 1994). The transcription factor NF-κB, a ubiquitously expressed transcription factor comprising a homo- or heterodimer of DNA-binding proteins related to the proto-oncogene c-*rel*, controls the expression of many immune- and inflammatory-response genes (Baeuerle and Henkel, 1994; Baeuerle and Baltimore, 1996). In most cells NF-κB exists in a latent state in the cytoplasm bound to inhibitory proteins (collectively called IκB) that mask its nuclear localization signal (Baldwin, 1996; Baeuerle, 1998). The latent form can be activated by inducing agents, including several cytokines that signal for phosphorylation and subsequent degradation of IκB.

Several adaptor proteins are involved in the events associated with this cytokine-induced effect, including the tumor necrosis factor receptor-associated factors (TRAF proteins) (Rothe *et al.*, 1994, 1995). One of them, TRAF2, binds to the p55 and p75 TNF receptors, as well as to several other receptors of the TNF/NGF receptor family, either directly or through other adaptor proteins. TRAF2 is crucial for the activation of NF-κB by these receptors, whereas TRAF6 is required for the induction of NF-κB by interleukin-1 (IL-1), a cytokine with activities similar to TNFα, although it used a structurally unrelated receptor. TRAF5 is involved in NF-κB activation by members of the TNF receptor family similar to TRAF2 (Akiba *et al.*, 1998). In contrast, TRAF6 participates in NF-κB activation by IL-1β (Liu *et al.*, 1996). TRAF6 associates with the serine-threonine kinase IRAK after the IL-1-induced activation of IRAK in the IL-1 receptor complex. The TRAF domain is involved in receptor association and homo- and hetero-oligomerization of TRAFs and serves as a docking site for a number of other signaling proteins (Takeuchi *et al.*, 1996). The NF-κB-inducing kinase (NIK) is one of such signaling proteins (Malinin *et al.*, 1997). NIK interacts with and activates the IκB kinases IKK-1/α, IKK-2/β, and IKK-3/γ/NEMO, which are part of a multiprotein IκB kinase complex, leading to the phosphorylation, ubiquitination, and subsequent degradation of the IκB (DiDionate *et al.*, 1997; Mercurio *et al.*, 1997; Zandi *et al.*, 1997; Yamaoka *et al.*, 1998).

Previously, antioxidant treatment or overexpression of endogenous redox molecule TRX was shown to block TNFα-mediated NF-κB activation by an unknown mechanism (Meyer *et al.*, 1993; Schenk *et al.*, 1994; Los *et al.*, 1995). To understand better how and at which level of the signaling cascades TRX interferes with the inflammatory cytokine-induced signal transduction leading to NF-κB activation, we used specific signaling proteins involved in this cytokine-induced NF-κB activation. Our results clearly demonstrate that TRX blocks TNFα or IL-1β-induced NF-κB activation at a step upstream of NIK.

MATERIALS AND METHODS

Cell lines and reagents

HEK293 and HeLa cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) (Gibco BRL Life Technologies Inc., Grand Island, NY) supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C under a humidified atmosphere of 5% CO_2.

Recombinant human TNFα and human IL-1β was from Boehringer Mannheim GmbH (Mannheim, Germany). The anti-human TRX monoclonal antibody, 11-mAb, was established and provided by FujiRebio, Inc. (Tokyo, Japan). Anti-p38 MAPK phospho-specific (Thr183/Tyr185) rabbit polyclonal antibody raised against a synthetic phospho-peptide (KLH coupled) corresponding to residues 172–186 of human p38 MAPK was purchased from New England Biolabs (Beverly, MA). Anti-p-JNK antibody (G-7) was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). *N*-acetyl-L-cysteine (NAC) and diphenyleneiodonium (DPI) were from Sigma (St. Louis, MI).

Expression plasmids

Mutagenesis of human TRX was performed by a PCR-based technique (Hirota *et al.*, 1997)

and subcloned into *Bam*HI/*Sal*I-cut pBluescript II SK+ plasmid (pBSII-TRX). Expression vectors for TRX, pcDNA3-TRX-wt and pcDNA3-TRX-C32/35S, were made by inserting a *Bam*HI–*Xho*I fragment from pBSII-TRX plasmids into *Bam*HI/*Xho*I-cut pcDNA3 (Invitrogen Corp., San Diego, CA). pcDNA3-anti-TRX was made by inserting a *Bam*HI–*Hin*dIII fragment from pcdSRαTRX plasmid into *Bam*HI/*Hin*dIII-cut pcDNA3. HA-tagged-TRAF2, TRAF5, TRAF6, and FLAG-tagged NIK were described elsewhere (Shinkura *et al.*, 1999). Active mitogen-activated protein kinase/ERK kinase kinase-1 (MEKK) (amino acids 360–672), pFC-MEKK, was from Stratagene. Hemagglutinin (HA)-tagged expression plasmids pBOS-HA-Rac1-V12(Gly12 to Val12) and -N17 (Thr17 to Asn17) were kindly provided by K. Kaibuchi (NAIST) and are described elsewhere (Kuroda *et al.*, 1996). Expression vectors of human ASK1, pcDNA3-ASK1K709R and pcDNA3-ASK1ΔN, were kindly provided by H. Ichijo (Saito *et al.*, 1998).

NF-κB reporter gene assay

A phagemid vector pNFκB-Luc, which contains five copies of the NF-κB-binding motif of the murine immunoglobulin κ light chain enhancer controlling luciferase expression, was purchased from Stratagene (La Jolla, CA). HEK293 cells were plated in 12-well plates at a density of 1×10^5 cells per well. The expression plasmids were introduced into the cells using Fugene 6 reagent (Boehringer Mannheim GmbH). In each transfection, expression plasmids, 0.5 μg of pNF-κB-Luc and 0.25 μg of pSV-β-galactosidase (Promega Corp., Madison, WI) as an internal control, were used. The total amount of DNA was adjusted equally with pcDNA3. After incubation for 16 hr, cells were stimulated with recombinant human TNFα or IL-1β for 6 hr. Then the cells were harvested and the luciferase activity was determined using a commercial assay system (Promega) with a luminometer, Lumat LB9507 (Berthold, GmbH & Co. KG, Bad Wildbad, Germany) (Hirota *et al.*, 1997). The relative fold induction of luciferase activity was calculated after normalization dividing the luciferase activity by β-galactosidase activity.

Western blotting

Each lysate was applied to a 15% SDS-PAGE and electrophoresed. After electroblotting, the PVDF membrane (Millipore, Bedford, MA) was treated with 5% (wt/vol) skim milk in TBS-T (20 m*M* Tris-HCl, pH 7.6, 137 m*M* NaCl, 0.5% Tween 20), and incubated with antigen-specific antibodies, followed by incubation with peroxidase-conjugated anti-immunoglobulin G (IgG) (Amersham Pharmacia Biotech, Uppsala, Sweden). The epitope was detected with an enhanced chemiluminescence (ECL) Western blot detection kit according to the manufacturer's instruction (Amersham Pharmacia Biotech).

RESULTS

Effects of TRX or antioxidant treatment on the NF-κB activation elicited by cytokines

In an attempt to explore redox regulation of NF-κB activation, we co-transfected HEK293 cells with an expression plasmid harboring various types of human TRX and a NF-κB reporter plasmid. As shown in Fig. 1, A and B, expression of wild-type TRX suppressed TNFα- or IL-1β-elicited NF-κB-dependent luciferase activity. This inhibition was a pcDNA3-TRX-wt-plasmid-dose dependent. In contrast, pcDNA3-TRX-C32/35S, lacking reducing activity (Hirota *et al.*, 1997), had no such suppressive effect at low dose (Fig. 1C). We found that higher level of expression of pcDNA3-TRX-C32/35S partially inhibited NF-κB activation by TNFα. Treatment with a cell-permeant thiol-based antioxidant NAC or DPI (Sundaresan *et al.*, 1996), a potent flavoprotein such as NADPH oxidase inhibitor suppressed luciferase activity as well as wild type TRX (Fig. 1B). Figure 1D showed that expression of the dominant-negative Rac1 mutant, Rac1-N17, inhibited TNFα- or IL-1β-stimulated NF-κB activities. Furthermore, transient expression of a constitutively active mutant of Rac1, Rac1-V12, induced NF-κB-dependent luciferase activity, and this stimulation was inhibited by co-expression of TRX-wt (Fig. 1E).

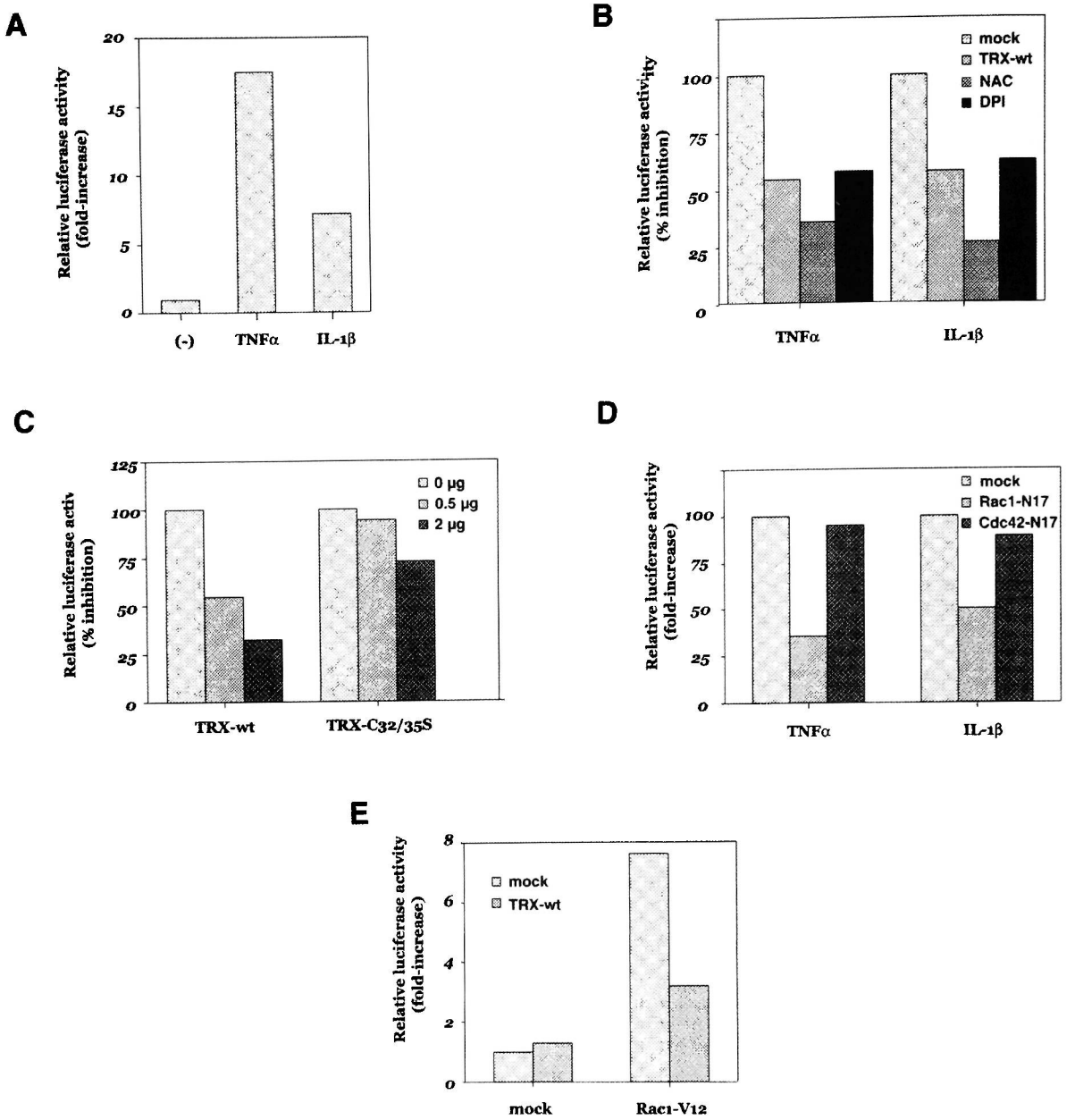

FIG. 1. Effects of TRX or antioxidant treatment on the NF-κB activation elicited by cytokines. HEK293 cells grown on 12-well plates were allowed to recover for 8–12 hr. After transfection, cells were incubated for 12 hr and then treated by TNFα (10 ng/ml) or IL-1β (20 ng/ml). After 6 hr, cells were harvested and subjected to the luciferase assay. In the case of NAC (10 m*M*) or DPI (5 μ*M*) treatment, cells were treated for 30 min before cytokine treatment. The dominant negative form (Rac1-N17 or Cdc42-N17) (**D**) or constitutively active form (Rac1-V12) (**E**) of the small G protein were cotransfected. The amounts of total plasmid were kept at 1.25 μg (**A** and **B**), 2.75 μg (**C**) or 1.75 μg (**D** and **E**). Cells were treated with 10 ng/ml of TNFα (**A, B, C,** and **D**) or 20 ng/ml of IL-1β (**A, B,** and **D**). The results are the means of three experiments (**A, B,** and **C**) or two (**E** and **D**) (each done in duplicate) and presented as fold-increases in luciferase activity over the baseline seen with the mock transfectant without treatment. The SDs were within not more than ±10% of the mean.

Effects of TNFα or IL-1β on the expression level of TRX

We further investigated a possible physiological involvement of TRX in cytokine-stimulated NF-κB activation. Previously, we reported that various oxidative stresses such as H_2O_2 (Taniguchi *et al.*, 1996), and UV irradiation (Sachi *et al.*, 1995) markedly induced TRX expression in cells. Expression levels of TRX in

HeLa cells 8 hr after treatment with TNFα or IL-1β were examined. In Fig. 2, TNFα or IL-1β significantly induced expression of TRX. This induction level is almost equal to that of introduction of pcDNA3-TRX-wt (Fig. 2; lanes 2, 3, and 4).

Transient overexpression of TRX or NAC treatment inhibited TRAF2, TRAF5, and TRAF6- but not NIK, MEKK, and IKKα-induced NF-κB activation in HEK293 cells

We next examined which molecules through the signaling cascade from the TNFα or IL-1β receptor to NF-κB activation are redox-sensitive. To explore this issue, we examined the effect of TRX expression or treatment with an antioxidant, NAC, on NF-κB activation by forced expression of the signaling molecules TRAF2, TRAF5, TRAF6, NIK, and IKKα (CHUK). Expression of TRAFs, NIK, or IKKα were sufficient to induce NF-κB-dependent gene expression (Fig. 3A). Reporter gene assays demonstrated that TRX inhibited NF-κB activation by all the tested TRAF molecules (TRAF2, TRAF5, and TRAF6), although the extents of inhibition were different from each other (Fig. 3B). NAC treatment (10 m*M*) also inhibited activation of the reporter gene by the TRAFs to a similar extent. Interestingly, in contrast to the TRAFs, neither TRX nor NAC inhibited NIK- or IKKα-induced NF-κB activation. MEKK1 is another MAPK kinase kinase (MAP3K) that induces the degradation of IκBα and activates NF-κB reporter gene (Lee *et al.*, 1998). NF-κB activation by a constitutively active form of MEKK1 was not blocked by either TRX or NAC (Fig. 3B).

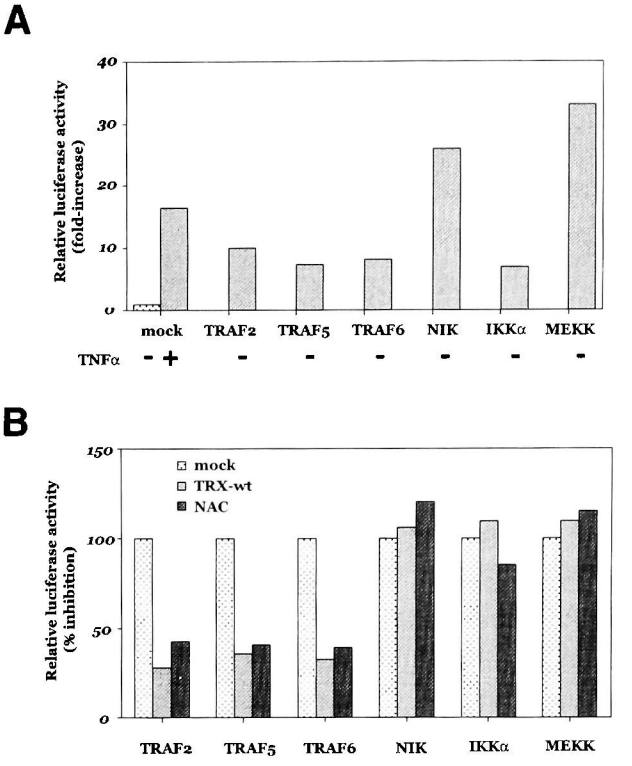

FIG. 3. Transient overexpression of TRX or NAC treatment inhibited TRAF2, TRAF5, and TRAF6- but not NIK, MEKK, and IKKα-induced NF-κB activation in HEK293 cells. HEK293 cells were transfected with expression plasmids of TRAF2, TRAF5, TRAF6, NIK, MEKK, and IKKα in pcDNA3 (**A**) or pcDNA3-TRX-wt (**A** and **B**). At 6 hr after transfection, NAC (10 m*M*) was added (**B**, right-most columns). At 12 hr after transfection, cells were harvested and subjected to the luciferase assay. The results are the means of three experiments (each done in duplicate) and presented as fold-increases in luciferase activity over the baseline seen with the mock transfectant without treatment. The SDs were within not more than ±10% of the mean.

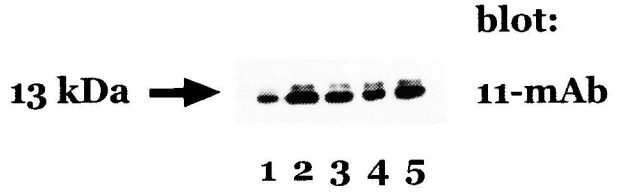

FIG. 2. Effects of TNFα or IL-1β on the expression level of TRX. HeLa cells were plated at a density of 3×10^5 cells per well onto multicluster (6×35-mm/well) plastic culture plates (IWAKI, Tokyo, Japan) and allowed to recover for 8–12 hr. Then, cells were treated with TNFα (100 ng/ml) (lane 2) or IL-1β (100 ng/ml) (lane 3) and incubated for 8 hr. In lanes 4 and 5, cells were transfected with pcDNA3-TRX-wt (lane 4, 0.5 μg, and lane 5, 2 μg), respectively, and incubated for 12 hr. Lane 1 is from the control sample. After treatment, cells were washed three times with ice-cold PBS and placed on ice. Cell lysates were prepared in SDS-sample buffer and quantified. A total of 5 μg of the lysates were subjected to Western blot analysis using the 11-mAb, anti-human TRX mouse monoclonal antibody as described in Materials and Methods.

TRX inhibited cytokine-induced NF-κB and p38 MAPK activation in a different manner

As shown in Fig. 4A, overexpression of wild-type TRX inhibited TNFα or IL-1β-induced p38 MAPK and SAPK/JNK phosphorylation in HEK293. In contrast, TRX-C32/35S, lacking reducing activity, had no inhibitory effect. To

know more regarding the molecular mechanism of inhibition of NF-κB activation by TRX, we examined the effects of ASK1 on NF-κB activation elicited by TNFα and IL-1β. Transient overexpression of ASK1ΔN (Saito *et al.*, 1998), the constitutively kinase-active mutant form, did not affect NF-κB activation by itself nor the cytokines-induced activation. ASK1K709R (Saito *et al.*, 1998), a kinase-inactive mutant form, did not influence cytokines-induced NF-κB activation.

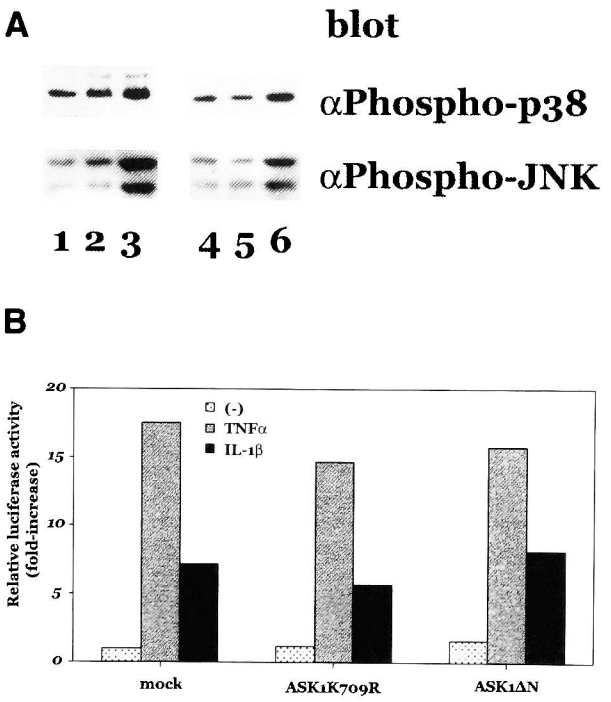

FIG. 4. TRX inhibited cytokine-induced NF-κB and p38 MAPK activation in different manners. A. HEK293 cells were transfected with pcDNA3 (lanes 1, 3, 4, and 6) or pcDNA3-TRX-wt (lanes 2 and 5) and were incubated for 24 hr. Next, cells were subjected to serum starvation. Six hours after, the cells were treated with 100 ng/ml TNFα (lanes 2 and 3) or 20 ng/ml IL-1β (lanes 5 and 6). In lanes 1 and 4, cells were not treated with any cytokines. After 15 min, cells were lysed and 20 μg of lysate was subjected to Western blot analysis. Phosphorylation of p38 MAPK was detected using phospho-specific p38 MAPK antibody (upper panel); phosphorylation of JNK/SAPK was detected using phospho-specific JNK antibody (lower panel), as described in Materials and Methods. **B.** HEK293 cells grown on 12-well plates were allowed to recover for 8–12 hr. After transfection with pcDNA3, or pcDNA3-ASK1(K709R), or pcDNA3-ASK1ΔN, cells were incubated for 12 hr and then treated with TNFα (10 ng/ml) or IL-1β (20 ng/ml). After 6 hr, cells were harvested and subjected to the luciferase assay. The results are the means of two experiments (each done in duplicate) and presented as fold-increases in luciferase activity over the baseline seen with the mock transfectant without treatment. The SDs were within not more than ±10% of the mean.

DISCUSSION

The transcription factor NF-κB becomes activated in several cell lines upon stimulation with the inflammatory cytokines TNFα and IL-1β, and is responsible for the transcription of many proinflammatory genes. Involvement of reactive oxygen intermediates (ROI) in its activation is suggested by many reports (Pahl and Baeuerle, 1994; Sen and Packer, 1996). Indeed, treatment with various antioxidants such as NAC or expression of TRX suppressed TNFα- and IL-1β-induced activation (Fig. 1A).

A variety of evidence suggests that nonphagocytic cells are capable of producing ligand-stimulated ROI. In fact, many of the components of the NADPH oxidase system appear to exist in a variety of cell types, and it is suggested that these systems are activated by the small guanosine triphosphate-binding protein Rac1 in nonphagocytic cells (Diekmann *et al.*, 1994; Prigmore *et al.*, 1995). Moreover, both growth factor- and cytokine-stimulated ROI production occurs through Rac1-dependent pathways (Sundaresan *et al.*, 1995; Sulciner *et al.*, 1996). The result shown in Fig. 1C, using a NADPH oxidase inhibitor, DPI, and in Fig. 1D, using a Rac1 mutant, suggests deep involvement of Rac1 in TNFα-elicited NF-κB activation. Results from experiments using a redox-inactive mutant suggest that the activity of TRX as an oxidoreductase may play an important role in this suppression. In fact, we and others have shown that TRX by itself (Mitsui *et al.*, 1992) or via other proteins such as peroxiredoxin (Prx) (Kang *et al.*, 1998a,b) and glutathione (GSH) peroxidase (GSH-Px) can reduce H_2O_2 as an essential intracellular second messenger. An alternative explanation could be that TRX influences the NADPH oxidase activity. Recently, using the yeast two-hybrid screening method, we identified p40phox, which is one of the proteins of the NADPH oxidase complex, as a TRX binding protein

(Nishiyama *et al.*, 1999). Although the involvement of p40phox in the NADPH oxidase system remains to be elucidated, TRX may play a regulatory role in this oxidase system via p40phox.

In Fig. 2, we showed that TNFα significantly induced TRX protein expression in HeLa cells. Taken together with the result of the experiment of forced expression of TRX, endogenous TRX induced by the cytokines may modulate the redox state in the cytoplasm and could work as an effector of negative feedback of NF-κB transcription in the physiological or pathophysiological circumstance.

Recently, the signal transduction pathways leading to NF-κB activation by TNFα and IL-1β have been largely elucidated (Baeuerle, 1998; Cohen *et al.*, 1998). Whereas the upstream proteins involved in these signaling pathways are different in the case of TNFα and IL-1β, both pathways converge at the NIK, a molecule of the MAP3K class. Expression of these signaling intermediates was sufficient to induce NF-κB-dependent gene expression (Fig. 3A). Taken together with the result from MEKK, it is suggested that MAP3K-induced NF-κB activation is not TRX sensitive. Our results clearly indicate that TRX interferes with the TNFα- or IL-1β-signal transduction pathway to NF-κB activation at steps upstream of NIK and IKKα, presumably at the level or immediate downstream of TRAFs. TRAF proteins consist of a conserved carboxy-terminal TRAF domain and an amino-terminal region containing a RING-finger motif and an additional array of zinc-finger-like structures (Takeuchi *et al.*, 1996). These zinc fingers are shown to be involved in self-association and interaction with other molecules in the TNF signaling cascade and contain reactive cysteines. These may be potential targets for redox regulation.

Apoptosis signal-regulating kinase (ASK) 1/MAPKKK5 was identified as one of the TRX-binding cellular proteins (Ichijo *et al.*, 1997). ASK1 was shown to be essential for signal transduction from the TNFα or the IL-1β receptor to SAPK/JNK (Nishitoh *et al.*, 1998), and mammalian TRX is reported to be a physiological inhibitor of ASK1 (Saito *et al.*, 1998). Our data presented here and described previously (Hashimoto *et al.*, 1999) are consistent with their reports. TRX and NAC is able to block TNF- and TRAF2-induced activation of JNK, suggesting that JNK activation by TNF and TRAF2 may be dependent on thiols/disulfides exchanging reactions. In this respect, it should be of particular importance that TRX inhibits TNF-induced activation of ASK1. It is suggested that the activation of ASK1 by TRAF2 might be regulated by the inactivation and subsequent dissociation of TRX from ASK1 (Saito *et al.*, 1998). However, in sharp contrast to the case of redox regulation of SAPK/JNK and p38, our results from the reporter assay clearly suggest that TRX inhibits cytokines-induced NF-κB activation by quite a different mechanism from that of MAPKs suppression. Interestingly, a high dose of pcDNA3-TRX-C32/35S partially suppressed NF-κB activation. It is reported that TRX-C32/35S is lack of reducing activity as radical scavenger and oxidoreductase. The inhibitory effect of high-dose expression may be a result of the dominant negative effect against oxidoreductase activity, as reported previously (Gallegos *et al.*, 1996), although we could not observe this effect in MAPK suppression. In fact, we recently demonstrated that TRX plays distinct roles in the cytoplasm and nucleus regarding NF-κB activation (Hirota *et al.*, 1999). Expression of TRX-C32/35S at high levels might inhibit the DNA-binding activity of NF-κB in HEK293 cells. The same molecule, TRX, and the same principle, redox, regulate very similar signal transduction systems differentially, although we cannot determine the underlying molecular mechanism exactly. Indeed, TRAF is the bifurcation point of two kinase cascades leading to activation of NF-κB and SAPK/JNK, respectively.

In summary, we have shown that TRX expression and antioxidant treatment suppress the inflammatory cytokines-induced NF-κB-mediated gene induction at the level of or downstream of TRAFs and upstream of the MAP3Ks; the redox activity of TRX is required for this inhibitory effect. Moreover, the results using mutant forms of ASK1 indicated that ASK1 is not involved in TRX-mediated NF-κB inhibition. This is in sharp contrast to the case of SAPK/JNK, in which ASK1 plays an essential role. TRAF2, TRAF5, and TRAF6 not only

link the TNF and IL-1 receptor to the NF-κB activation cascade, but have also been suggested to be involved in the NF-κB activation by CD40, the human Toll-like receptor, and some other members of the TNF and IL-1 receptor superfamily. TRX may play an important role in these signaling cascades.

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ABBREVIATIONS

ASK, Apoptosis signal-regulating kinase; DMEM, Dulbecco's modified Eagle medium; DPI, diphenylene iodonium; ECL, enhanced chemiluminescence; GSH, glutathione; HA, hemagglutinin; H_2O_2, hydrogen peroxide; IgG, immunoglobulin G; IL-1, interleukin-1; MEKK, mitogen-activated protein kinase/ERK kinase kinase-1; NAC, *N*-acetyl-L-cysteine; NIK, NF-κB-inducing kinase; NO, nitric oxide; Prx, peroxiredoxin; ROI, reactive oxygen species; TNF, tumor necrosis factor; TRAF, TNF-receptor-associated factors; TRX, thioredoxin.

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