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Induction of TNFα in macrophages by vanadate is dependent on activation of transcription factor NF-κB and free radical reactions

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Abstract

Vanadium-induced TNFα production is believed to play an important role in respiratory disease associated with air pollution and occupational exposure. While vanadium is able to induce TNFα in macrophages or airway epithelial cells, the underlying mechanism is not well defined. In the present study, mechanisms of vanadate-induced TNFα production were analyzed in the murine Raw264.7 cells. Vanadate induces a significant amount of TNFα at both the protein and mRNA levels, and the induction is vanadate dose-dependent. The mechanism analysis was focused on transcriptional regulation of TNFα gene by vanadate. Transient transfection studies show that the TNFα gene promoter was activated by vanadate and this activation was associated with an increase in DNA binding activity of the nuclear factor-κB (NF-κB). Mutation of the NF-κB binding site in the gene promoter led to a loss of the promoter responsiveness to vanadate, indicating requirement of NF-κB. This is supported by evidence that inhibition of NF-κB activation by SN50, a specific NF-κB inhibitor, resulted in a decrease in the TNFα production. A role of reactive oxygen species (ROS) was explored in vanadate activity. The result shows that vanadate-induced TNFα production is elevated by NADPH, which enhances vanadate-mediated generation of ROS, but is inhibited by an antioxidant, N-acetyl-L-cysteine (NAC). Modification of TNFα production is associated with an enhancement or a repression of NF-κB activity by NADPH or NAC, respectively. Taken together, these results indicate that: (a) activation of the TNFα gene promoter contributes to the vanadate-induced TNFα production; (b) NF-κB is required for the vanadate-induced promoter activity of TNFα gene; (c) free radical reactions are involved in the vanadate-induced TNFα production and NF-κB activation. (Mol Cell Biochem **198**: 193–200, 1999)

Key words: TNFα, vanadate, NF-κB activation, reactive oxygene species

Introduction

Inflammatory cytokines induced by vanadium are involved in the pathological mechanisms of the air pollution-related respiratory disease and mortality [1, 2]. TNFα is one of the most important inflammatory cytokines involved in the occurrence of acute or chronic respiratory diseases. Although many types of cells are able to produce TNFα, cells of monocytic lineage are the primary producers. In the lung, macrophages are able to release inflammatory mediators, including TNFα, upon exposure to particles, such as silica [3, 4] or asbestos [5, 6]. In the animal studies, vanadium compounds or vanadium-containing air pollution particles have been shown to induce inflammation in the respiratory tract [1, 2]. Although vanadate was demonstrated to induce TNFα production and result in inflammation in these studies, the underlying mechanism of vanadium-induced TNFα production remains to be studied.

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While expression of TNFα is regulated at several levels: Transcriptional, post transcriptional, translational, and post-translational [7], it is generally accepted that the induction of TNFα is mainly controlled by transcription. The transcription factor, NF-κB, is one of the nuclear proteins that governs the initiation of TNFα transcription [8–10]. Other transcription factors that contribute to the activation of the TNFα promoter include the activator protein 1 (AP-1) [11], the nuclear factor of activated T cells (NFAT) [12], the early growth-response transcription factor 1 (Egr-1) [13], the cAMP response element binding protein (CEBP) [14], the CATT enhancer binding protein beta (C/EBPβ) [15], the activated protein 2 (AP-2) [11, 16] and Ets [17]. The role of each transcription factor in the regulation of TNFα promoter is dependent on the cell types [18] and the nature of stimulation [17, 19]. For example, NF-κB, but not AP-1 or AP-2, is the major activator of TNFα transcription in monocytes stimulated by lipopolysaccharide [10]. In the PMA-stimulated T cells, NFAT, not NF-κB, plays a major role [19].

Although vanadium or vanadium-containing particles are able to induce TNFα in several types of cells including human monocytes [1, 20–22], it is not clear whether vanadate induces the gene expression at the transcriptional level. In the present study, the vanadium-induced TNFα production was analyzed in a macrophage cell line with a focus on the gene transcription and a role of free radicals in the activity of vanadate. This study provides the first evidence that vanadate activates the TNFα gene promoter and the activation is mediated by the NF-κB binding site. The results also demonstrate that activation of NF-κB is required for the induction of TNFα by vanadate, and reactive oxygen species (ROS) mediate both TNFα induction and NF-κB activation induced by vanadate.

Materials and methods

Cells and reagents

The mouse monocyte-macrophage cell line Raw 264.7 was purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in DMEM supplemented with 10% fetal calf serum [FCS], 2 mM glutamine and 100 U/mi penicillin-streptomycin (complete medium). A specific antibody against the NF-κB p50 subunit (Cat # SC11 4x) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and used in the supershift assay. Sodium metavanadate (Cat. # 28939-1) was purchased from Aldrich Chem. Co (Milwanka, WI, USA). β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH) (Cat.# N-1630) and N-acetyl-L-cysteine (NAC, Cat.# A7250) were purchased from Sigma Chemical Company (St. Louis, MO, USA). These reagents were freshly prepared in 1 × PBS as a stock solution of 20 fold and kept at 4°C. SN50 (Cat # p-600) was obtained from BIOMOL Research Laboratories (Plymouth Metting, PA, USA).

TNFα ELISA assay

Macrophage cells (1×10^5/well) were plated in a 96 well plate for 4–16 h before stimulation. Sodium metavanadate in PBS was added to the cells at a final concentration indicated in each figure legend. NAC or SN50 was preincubated with the cells for 30 min to inhibit vanadate-induced TNFα production or NF-κB activation. Three wells were used in each treatment. The cell culture supernatant was harvested at the end of treatment, combined together from the tripled wells and used for TNFα assay. An ELISA kit (Cat # 802802-5) from the Genzyme Corporation (Cambridge, MA, USA) was used to determine the TNFα level in the supematant according to the manufacturer's instruction.

Messenger RNA (mRNA) assay

The total RNA was extracted from cells using the RNeasy mini kit (QIAGEN, Hilden, Germany). The TNFα mRNA was analyzed by reverse transcriptase PCR (RT-PCR). Quantitation of mRNA was conducted with the competitive RT-PCR techniques using the PCR MIMIC template (Clontech, CA, USA) as an internal control for the murine TNFα according to the manufacturer's instructions.

Gel shift assay

A NF-κB binding sequence in the human IL-6 gene promoter (-74 TGGGATTTTCCCATGAGTCT -54) was used to synthesize an oligonucleotide for the NF-κB probe [23]. The complementary single-stranded oligonucleotides were denatured at 80°C for 5 min and annealed at room temperature. An AP-1 binding oligonucleotide derived from the AP-1 binding sequence in the collagenase gene promoter was used as a nonspecific competitor or as a probe to examine the AP-1 binding activity [24, 25]. The double-stranded probe was labeled with ^{32}P-ATP (Amersham, Arlington Heights, IL, USA) using the T4 kinase (BRL, Gaithersburg, MD, USA). The nuclear extracts were prepared as follows: 5×10^7 cells were treated with 500 µl lysis buffer (50 mM KCl, 0.5% NP-40, 25 mM HEPES pH 7.8, 1 mM PMSF, 10 µg/ml Leupeptin, 20 µg/ml Aprotinin, 100 µM DTT) on ice for 4 min. After 1 min centrifugation at 14,000 rpm, the supernatant was saved as a cytoplasmic extract. The nuclei were washed once with the same volume of buffer without NP-40, then were treated with 300 µl of extraction buffer (500 mM KCl, 10% Glycerol with the same concentrations of HEPES, PMSF, Leupeptin, Aprotinin and DTT as the lysis buffer) and pipetted several

times. After centrifugation at 14,000 rpm for 5 min, the supernatant was harvested as the nuclear protein extract and stored at –70°C. The protein concentration was determined using BCA protein assay reagent (Pierce, Rockford, IL, USA).

The DNA-protein binding reaction was conducted in a 24 µl reaction mixture including 1 µg Poly dI.dC, 3 µg nuclear protein extract and 3 µg BSA, 4×10^4 cpm of ^{32}P-labeled oligonueleotide probe and 12 µl of reaction buffer (24% glycerol, 24 mM HEPES pH 7.9, 8 mM Tris-HCl, pH 7.9, 2 mM EDTA, 2 mM DTT) [26]. In some cases, the indicated amount of double stranded oligomer was added as a cold competitor. The reaction mixture was incubated on ice for 10 or 20 min (with antibody) in the absence of radiolabeled probe. After addition of the radiolabeled probe, the mixture was incubated for 20 min at room temperature, then resolved on a 5% acrylamide gel that had been pre-run at 170 V for 30 min with 0.5 × TBE buffer. The loaded gel was run at 200 V for 90 min, then dried and placed on Kodak X-ON1AT film (Eastman Kodak, Rochester, NY, USA). The film was developed after an overnight exposure at –70°C.

Transfection assay

Two reporter gene vectors used in this study were gifts from Dr. S. T. Fan at the Scripps Research Institute (La Jolla, CA, USA) [10]. The wild type TNFα luciferase vector contains a promoter fragment (–615/+15) of the human TNFα gene. The mutant type TNFα luciferase vector is derived from the wild type luciferase vector by point mutation of the NF-κB binding site (κB3 site) in the TNFα promoter. The murine macrophage cells (1×10^6/well) were plated in a six well plate for 16 h before transfection. The reporter DNA (5 µg) was used in the transfection using DEAE-Dextran method [24]. After transfection, the cells were washed once in PBS solution and cultured in 3 mi of the complete medium at 37°C for 24 h. After stimulation for additional 16 h, the cells were harvested for the reporter assay. The luciferase activity was determined using an assay kit (Promega, Madison, WC, USA) then normalized for protein content. A mean value from 3 individual experiments was analyzed by the Student's *t*-test at a confidence level $p < 0.05$–0.001.

Results

Vanadate induces a dose-dependent production of TNFα protein in macrophages

The time course study showed that although a 6 h stimulation of Raw 256 cell line with sodium metavanadate could result in a significant enhancement of TNFα production (data not shown), a 16 h stimulation caused a higher yield of TNFα production. Thus, a stimulation of 16 h was used in the subsequent experiments. The concentration-response relationship for vanadate was examined with a 16 h stimulation (Fig. 1). The results showed that a minimum concentration of 40 µm is required for the induction of TNFα. The vanadate treatment resulted in a linear increase in the TNFα production over the range of 20–100 µM of vanadate.

TNFα induction was also examined at mRNA level. The total cellular RNA was prepared after the cells were treated with 60 µM of sodium metavanadate for 1 h. The TNFα mRNA was quantitated in the competitive RT-PCR using the MIMIC protocol (Fig. 1B). The MIMIC DNA served as an internal control for the TNFα signal (Fig. 1B). Quantitation of the interested gene product is determined by dilution of the internal control to identify a point where the two PCR

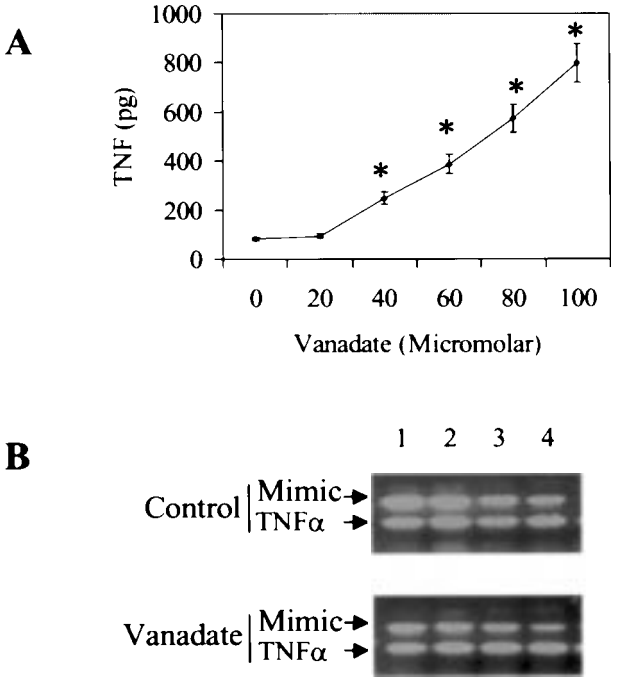

Fig 1. Induction of TNFα production in the murine Raw 264.7 cells. (A) Dose-dependence of vanadate-induced TNFα protein. The cells were treated with different doses of vanadate as indicated. The TNFα level in the cell supematant was determined with an ELISA as stated in the Materials and methods. Each point represents a mean value and standard deviation of three independent experiments. "*" indicates a significant ($p < 0.001$) stimulation of TNFα production by vanadate. (B) Quantitation of TNFα mRNA by RT-PCR. One microgram of total cellular mRNA was used in synthesis of the first strand cDNA in the reverse transcription. One out of 50 parts of the product was used in the competitive PCR reaction. The internal control template (Mimic) was diluted sequentially twofold at a time from 1:400 to 1:3200 in lanes one through four to identify a dilution where the amount of Mimic equals the TNFα PCR product. The unstimulated cells (control cells) reached this point at a dilution of 1: 1600 of the internal control (lane 3), while the vanadate-treated cells reached the equality point at a dilution of 1:400 (lane 1).

products become equal. The product of unstimulated cells reached this point at a dilution of 1:1600 of the internal control (Fig. 1B, lane 3), while the vanadate-treated cells reached the equal point at a dilution of 1:400 (Fig. 1B, lane 1). These results indicate that the TNFα mRNA level in the vanadate-treated cells is 4-fold of that in the control cells.

Induction of TNFα by vanadate is associated with activation of transcription factor NF-κB

The DNA binding activity of NF-κB in the vanadate-treated cells was investigated because the cytokine induction occurs predominantly at the transcriptional level, and NF-κB is a major activator for TNFα transcription in macrophages. The DNA binding activity of NF-κB was analyzed in the nuclear extract of vanadate-treated cells using the gel shift assay. The result show that the NF-κB activity was induced in the nuclear extract from cells treated by vanadate (Fig. 2A). The nuclear proteins formed a typical pattern of NF-κB bands, the upper p65/p50 heterodimer band and the lower p50/p50 homodimer band. The two NF-κB complexes were confirmed in the oligonucleotide competition and antibody supershift assay (Fig. 2B). As both the upper band and lower band are enhanced by vanadate, the results suggest that vanadate induced the binding activities of both p65 and p50 subunits in the NF-κB complexes. This increase was directly related to the vanadate concentration over the range of 40–100 μM. It may be noted that although the IL-6 NF-κB probe was utilized for the measurement of NF-κB activation, it is expected that the same results will be obtained using TNFα NF-κB probe. Activated NF-κB protein will bind to any established NF-κB site.

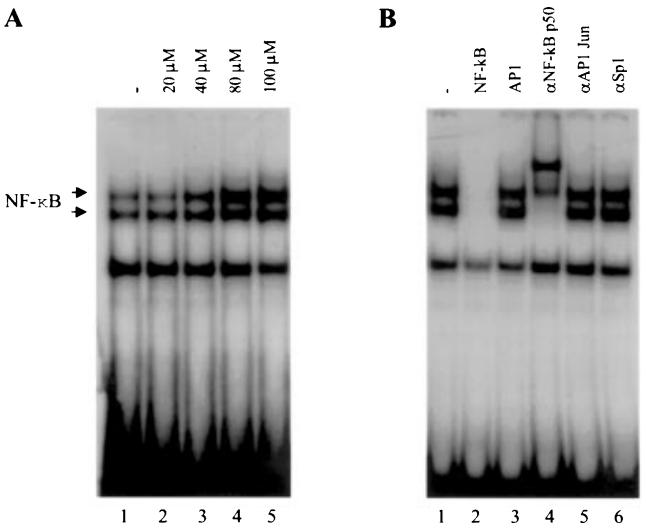

Fig. 2. DNA binding activity of transcription factor NF-κB in the nuclear extract of macrophages treated with vanadate. (A) Dose-dependence of NF-κB activity in the vanadatetreated cells. The DNA-binding activity of NF-κB was examined in the nuclear extract of vanadate-treated cells as stated in the Materials and methods. (B) Characterization of NF-κB complexes in the oligonucleotide competition assay and antibody supershift assay. The nuclear protein of cells treated with 100 μM vanadate was used with the NF-κB probe to form the DNA-protein complexes. Lane 1 served as a control for the oligonucleitide competition assay. The unlabeled NF-κB probe (100 μg) was used in Lane 2 as a specific competitor. The same amount of unlabeled AP-1 probe was used in the lane 3 for a nonspecific competition. The antibody against the p50 subunit of NF-κB protein was added in Lane 4 to confirm the protein nature of DNA-protein complexes. Lane 5 and 6 contained 200 μg of antibody against Spl and the c-Jun subunit of AP-1 protein, respectively, to serve as nonspecific antibodies.

Requirement of NF-κB in the activation of TNFα gene promoter by vanadate

The above results suggest that the induction of NF-κB activity may be responsible for the TNFα gene transcription. The promoter activity of the TNFα gene was examined in a transient transfection assay after stimulation with vanadate. The TNFα promoter-containing reporter plasmid was used to transfect the cells and the reporter activity was examined 48 h later. The results showed that vanadate induced a strong activity in the wild type TNFα gene promoter (Fig. 3, wild), but failed to induce a promoter response with the mutant

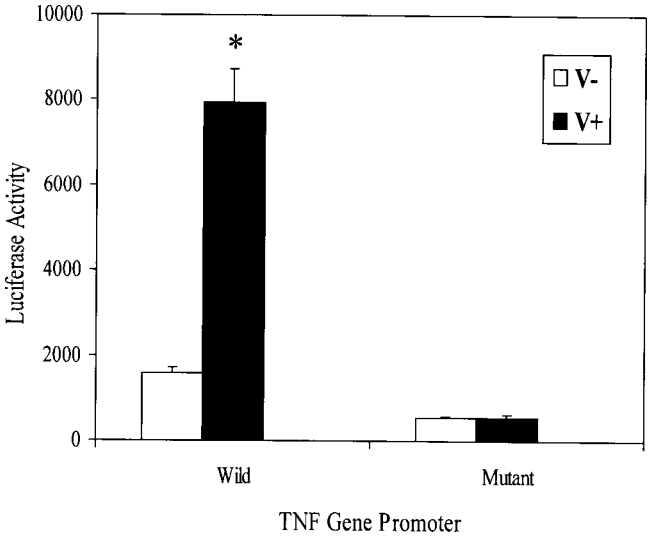

Fig. 3. Vanadate-induced promoter activity of TNFα in the transient transfection assay. The luciferase reporters controlled by wild type (wild) or mutant (mutant) TNFα promoter were transfected into the cells. The cells were treated with 80 μM vanadate for 16 h. The reporter activity in the cell lysate was determined using the luminometer and the reading was normalized for the amount of protein used in each reporter assay. Each bar represents a mean value and standard deviation of results from three individual transfections. "*" indicates a significant ($p < 0.001$) stimulation by vanadate.

promoter in which the NF-κB binding site was mutated (Fig. 3, mutant). The difference indicates that mutation of the NF-κB binding site in the TNFα promoter DNA leads to a loss of response of the gene promoter to vanadate. These results demonstrate that vanadate is able to activate the TNFα gene promoter and that the NF-κB binding site in the promoter is required for transcriptional activation by vanadate.

A NF-κB specific inhibitor, SN50, was used to further examine the role of NF-κB in the induction of TNFα by vanadate. SN50 is a small peptide containing a cell membrane permeable amino acid sequence and a translocation signal of the NF-κB p50 subunit [27]. This agent can specifically block translocation of NF-κB from the cytoplasm into the nucleus. The result showed that addition of SN50 to the cell culture resulted in a remarkable inhibition of TNFα response to vanadate (Fig. 4A). This inhibition was associated with a reduction in the DNA binding activity of NF-κB (Fig. 4B), suggesting that blockage of NF-κB activity is responsible for the inhibition of TNFα production. The NF-κB binding site is required for the vanadate-induced promoter activity (Fig. 3) and blockage of NF-κB translocation leads to the inhibition of TNFα production (Fig. 4). These data indicate that the activation of NF-κB is required for vanadate-induced TNFα expression. It may be noted that SN50 can inhibit basal level of TNFα. This is due to that SN50 can inhibit basal activity of NF-κB in Raw cells (data not shown). This basal NF-κB activity can be seen in Fig. 2 (Lane 1).

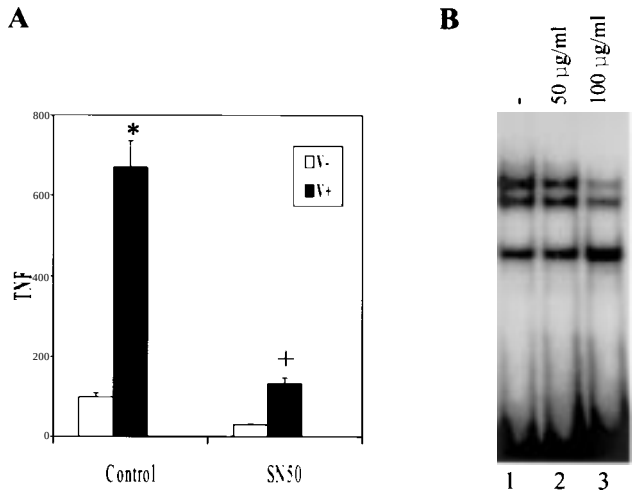

Fig. 4. Inhibition of TNFα production and NF-κB activation by SN50. (A) SN50 (100 μg/ml) was used to treat the cells in a 96 well plate and 80 μM vanadate was used to induce TNFα. Each bar represents a mean value of TNFα (pg) from three independent experiments. "*" indicates a significant ($p < 0.001$) increase from control while "+" indicates a significant ($p < 0.001$) inhibition of the vanadate response by SN50. (B) The nuclear protein was made from 5×10^6 cells after treatment of SN50 (dose indicated) plus vanadate (80 μM) for 3 h. The DNA binding activity of NF-κB in the nuclear protein was determined in the gel shift assay as stated in Materials and methods.

Activation of NF-κB is associated with vanadate-induced free radical reactions

Activation of NF-κB can be mediated by redox reactions in cells [28–31]. While reactive oxygen species (ROS) play an important role in vanadate-induced biological responses [32], it has not been clarified whether ROS play a role in induction of TNFα by vanadate. To examine the possible role of free radical reactions in the mechanism of vanadate-induced TNFα production, NADPH was used to enhance the generation of ROS in the vanadate-treated cells [33, 34]. TNFα production and NF-κB activation were monitored after an addition of NADPH to the cells. The results showed that NADPH alone did not stimulate TNFα production, but it dramatically augmented vanadate induced TNFα production (Fig. 5A). The enhancement was about three-fold at all concentrations (20–100 μM) of vanadate tested (data not shown). In the presence of NADPH, even 20 μM vanadate was able to induce TNFα production to a level equivalent to that induced by 60 μM vanadate alone. NADPH also enhanced the vanadate-induced DNA-binding activity of NF-κB (Fig.

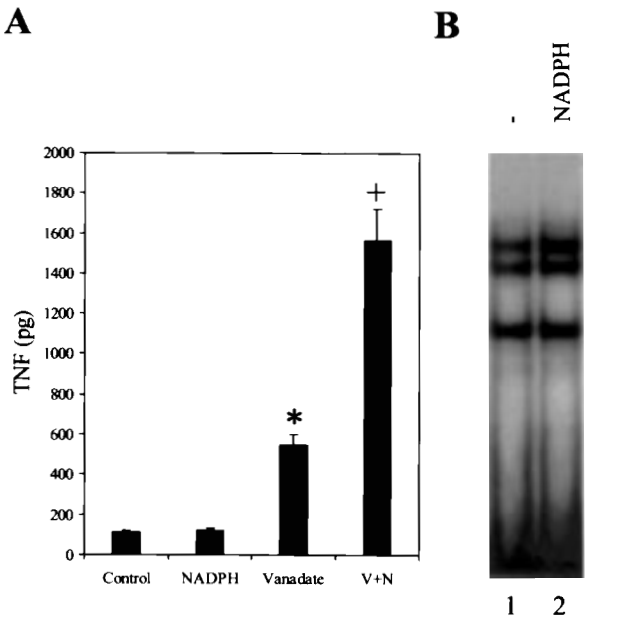

Fig. 5. Enhancement of vanadate-induced TNFα production and NF-κB activity by NADPH. (A) Enhancement of vanadate-stimulated TNFα production by NADPH (0.5 mM). The TNFα level in the cell supematant was determined after 16 h of stimulation. Treatment is indicated under each bar. "V+N" stands for vanadate plus NADPH. Each bar represents a mean value of three independent experiments. "*" indicates a significant ($p < 0.001$) increase from control, and "+" indicates a significant increase from vanadate alone. (B) Enhancement of NF-κB activity by NADPH. The nuclear protein was made from 5×10^6 cells treated with a combination of 80 μM vanadate plus 0.5 mM NADPH for three hours. The DNA binding activity of NF-κB in the nuclear protein was determined in the gel shift assay as stated in Materials and methods.

5B). The nuclear proteins from cells treated with vanadate alone (Lane 1) or vanadate plus NADPH (Lane 2) were examined in the gel shift assay. The result showed that it enhanced vanadate-induced activation of NF-κB. NADPH alone did not activate NF-κB (data not shown). The results demonstrate that an increase in the generation of reactive oxygen species may be responsible for the observed synergistic effect of NADPH and vanadate. It may be noted that the degree of NADPH-enhanced NF-κB activation is relatively weak compared with the NADPH-enhanced TNFα production. This may be due to the fact that there are four NF-κB binding sites in the mouse TNFα promotor, and synergism among these four sites may boost activity of NF-κB that has already been enhanced by NADPH.

N-acetyl-L-cystein (NAC) was utilized to examine the role of ROS in the mechanism of vanadate-induced TNFα production and NF-κB activation. NAC is an established antioxidant that has been shown to block NF-κB activation by ROS [28, 29]. Effects of NAC were determined by both TNFa production and NF-kB gel shift assays. The results show that 20 mM of NAC significantly inhibited both TNFa production (Fig. 6A) and NF-kB activation induced by vanadate (Fig. 6B), further supporting the role of ROS in the action of vanadate. NAC (20 mM) alone did not show any toxic effect on the cells as observed by typen blue staining (data not shown).

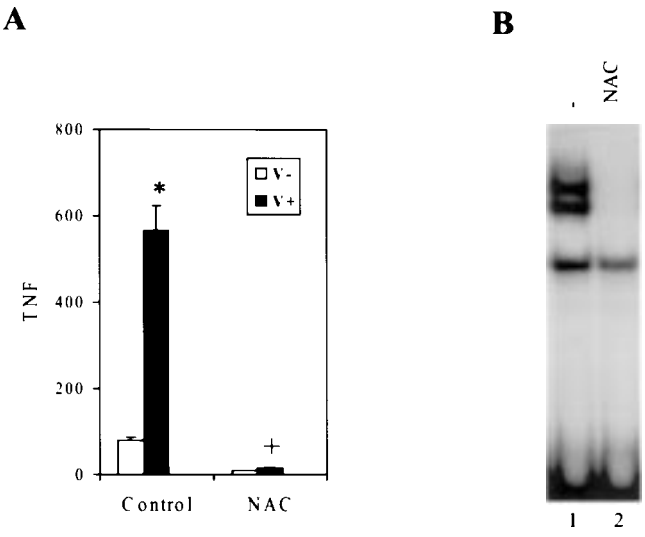

Fig. 6. Inhibition of vanadate-induced TNFα production and NF-κB activation by NAC. (A) NAC (20 mM) was used to treat the cells in the presence of 80 μm vanadate. The TNFα level in the cell supematant was determined after 16 h of combined treatment. Each bar represents a mean value of TNFα (pg) from three independent experiments. "*" indicates a significant ($p < 0.001$) stimulation with vanadate while "+" indicates a significant ($p < 0.001$) suppression of the vanadate response with NAC. (B) The nuclear protein was made from 5×10^6 cells treated with a combination of 80 μM vanadate and 20 mM NAC for 3 h. The DNA binding activity of NF-κB in the nuclear protein was determined in the gel shift assay as stated in Materials and methods.

Discussion

As mentioned in the introduction, vanadium is an important component of air-emission particles, such as residual oil fly ash in the polluted air. Experimental studies have demonstrated that vanadium-containing particles are able to induce inflammation in the airway of the respiratory system [1, 2]. TNFα is one of the pro-inflammation cytokines induced by these particles. In the present study, the molecular mechanism of vanadium-induced TNFα was investigated in macrophages. The results indicated that vanadate is able to induce TNFα at both the protein and mRNA levels and ROS are involved in the TNFα gene activation via NF-κB activation.

The molecular events of vanadium-induced TNFα expression are not well understood, and is the focus of the present study. The NF-κB binding sites are critical for the transcriptional activation of the TNFα gene in macrophages. This has been demonstrated in both human [10–35] and murine [8, 9] TNFα gene promoters. The present study shows that stimulation of macrophages with vanadate resulted in a dose-dependent increase in DNA-binding activity of NF-κB. This result is consistent with recent reports that vanadium is able to activate the DNA-binding activity of NF-κB in other type of cells [36–38].

The results obtained from the present study suggest that activation of transcription factor NF-κB is required for the vanadate-induced TNFα gene expression. The following observations support the role of NF-κB in vanadate-induced TNFα gene transcription. First, in the transfection assay of macrophages, we provide first evidence that vanadate can enhance the promoter activity of TNFα gene, and this enhancement is dependent on the presence of a NF-κB binding site in the gene promoter. Second, an increase in the NF-κB activation by NADPH is associated with an elevated level of TNFα production in the vanadate-treated macrophages. Third, blockage of NF-κB activation by SN50 or NAC resulted in the inhibition of TNFα induction by vanadate.

The role of ROS in the mechanism of vanadate-induced TNFα production was also examined. The results demonstrated that free radicals reactions are involved in the NF-κB activation and TNFα production induced by vanadate in macrophage. It should be noted that two signaling pathways have been proposed for NF-κB activation by vanadium [37]: (a) free radical-dependent pathway and (b) free radical-independent pathway. The role of redox reactions in the activation of NF-κB has been shown to vary with cell type [39]. For example, the oxidative stress plays a role in NF-κB activation by okadaic acid (a serine-threonine phosphatase inhibitor) in macrophages, but experiments using T cells (Jurkat) and fibroblasts (Hela) yielded contradictory results [40, 41]. It has been reported that in the T cells, vanadate is able to result in phosphorylation of IκB and activation of NF-κB [36, 37]. Although the antioxidant NAC was able to

block the phosphorylation of IκB, it failed to inhibit the activation of NF-κB [37]. This suggests that in the T cells, vanadate induces NF-κB through a free radical independent pathway. The results obtained in the present study indicate that in macrophages, activation of NF-κB by vanadate depends on the generation of oxidant species. This conclusion is supported by the following facts: (A) NADPH, which enhances vanadate generation of ROS, significantly enhanced vanadate-induced NF-κB activation. It has been reported that in the presence of NADPH, several flavoenzymes, such as the glutathione reductase, are able to cause one-electron reduction of vanadate [33]. In the reduction process, molecular oxygen is reduced to superoxide, which generates H_2O_2 via dismutation. Vanadium (IV) reacts with H_2O_2 to generate ·OH radical [34]. This radical is the most reactive among ROS and has been proposed as a messenger for NF-κB activation [42, 43]. (B) NAC, an antioxidant, blocked both NF-κB activation and TNFα production induced by vanadate, further supporting the role of ROS in the NF-κB activation. Combined together, these results suggest that activation of NF-κB and the resultant TNFα production by vanadate is mediated by ROS.

In conclusion, the results obtained from the present study shows that: (1) vanadate is able to induce TNFα production in macrophages; (2) transcriptional activation of the TNFα gene promoter contributes to this cytokine expression induced by vanadate; (3) activation of the transcription factor NF-κB, which is responsible for the induced activity of TNFα gene promoter, is required for the TNFα production induced by vanadate; (4) free radical reactions are involved in both vanadate-induced TNFα production and activation of NF-κB in macrophage.

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