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Original Contribution

NUCLEAR FACTOR-κB MEDIATES OVER-EXPRESSION OF CYCLOOXYGENASE-2 DURING ACTIVATION OF RAW 264.7 MACROPHAGES IN SELENIUM DEFICIENCY

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Abstract—Selenium (Se) is an essential micronutrient for all mammalian species and is associated with a variety of physiological functions, notably immune system, in the form of selenoproteins. Inadequate Se nutrition has been linked to various diseases, including rheumatoid arthritis, cardiomyopathy, and cancer. Important to this discussion is that cyclooxygenase-2 (COX-2) is over-expressed in all the aforesaid pathologies; however, a casual relationship between Se status and COX-2 expression remains to be established. The present study is based on the hypothesis that oxidant stress, a consequence of Se deficiency, lowers the activation potential of the redox-sensitive transcription factor, NF-κB, and that the activated NF-κB is required for the altered expression of COX-2. To test this hypothesis, we have investigated the relationship between Se status and COX-2 expression in response to LPS stimulation in RAW 264.7, a macrophage-like cell line. In Se-deficient cells, the Se-dependent glutathione peroxidase activity (Se-GPx), a measure of Se status, was markedly reduced and the overall oxidative stress was significantly higher than Se-supplemented cells. Upon lipopolysaccharide (LPS) stimulation, we found 2-3-folds higher COX-2 protein expression as well as higher PGE_2 levels in Se-deficient cells than Se-supplemented cells. In comparison, COX-1 protein expression was not affected by either LPS stimulation or Se status. Following LPS stimulation, the nuclear localization of NF-κB was significantly increased in Se-deficient macrophages, thereby leading to increased expression of COX-2. This is the first report demonstrating an inverse relationship between Se status and the expression of COX-2. © 2002 Elsevier Science Inc.

Keywords—Selenium deficiency, Glutathione peroxidase, Prostaglandin E_2, Cellular oxidative stress, Free radicals

INTRODUCTION

Selenium (Se) is an essential micronutrient for all mammalian species and functions primarily through selenoproteins, which contain Se as selenocysteine (SeCys). These selenoproteins have different physiological functions, but for those in which the function is known, SeCys is located at the active center [1,2]. The most well-characterized selenoenzymes are the Se-dependent glutathione peroxidases (Se-GPxs) and thioredoxin reductase (TrxR) families, the activities of which are responsible for the recognition of Se as an important dietary antioxidant [1]. These enzymes are involved in many biochemical processes supporting life. The most important processes include protection against oxidative stress and redox-based regulation of gene expression [2]. Selenium deficiency results in a significant decrease in Se-GPx and TrxR, and an increase in reactive oxygen species (ROS) production [1,2]. Inadequate Se nutrition and associated ROS production have been linked to increased risk of such diseases as cardiomyopathy [3], rheumatoid arthritis [4], and, most notably, cancer [5,6]. Emerging evidence from clinical trials and epidemiological studies has shown the beneficial effects of Se supplementation in prevention and/or treatment of some of these diseases [7]. For instance, studies have demonstrated the chemopreventive activity of Se against the development of tumors or cancers in both animal models and humans [8–10]. Furthermore, it has been documented that the level of Se in cancer patients is lower

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than that in normal subjects [11]. However, the molecular mechanisms contributing to the anticarcinogenic and chemopreventive activity of Se are not very clear and are the current topics of intense investigation.

Cyclooxygenase (COX), also known as prostaglandin H synthase (PGHS), is the rate-limiting enzyme in prostaglandin (PG) biosynthesis [12]. There are two isoforms of this enzyme: the constitutive form, COX-1, which is implicated in housekeeping or homeostatic functions, and COX-2, which is an inducible form in response to injury or inflammation [12]. The discovery of COX-2 in the early 90s was followed by an unprecedented search for drugs, which specifically inhibit COX-2, with no influence on COX-1. Intensive research on these isozymes has revealed distinct differences in their function and regulation [12]. Cyclooxygenase-2 can be induced by various pathogenic stimuli, including proinflammatory cytokines, mitogens, bacteria, and ROS [13]. In addition, there is a growing body of evidence to suggest that the nuclear factor-kappa B (NF-κB), a well-known redox-sensitive transcription factor, is involved in the regulation of COX-2 induction by various stimuli [14,15]. Important to this discussion is the fact that COX-2 is over-expressed in inflammatory diseases as well as several types of human cancers, and COX inhibitors have been shown to be anti-inflammatory and chemopreventive [16,17].

In the present study we have investigated the relationship between Se status and COX-2 expression in response to LPS stimulation in RAW 264.7, a murine macrophage-like cell line. These cells have been previously established as a model for the study of COX-2 regulation [18]. Our studies are based on the hypothesis that inadequate Se status in macrophages leads to an oxidant-antioxidant imbalance, and this imbalance leads to a lowered activation potential for the redox-sensitive transcription factor, NF-κB, which, in turn, induces the expression of COX-2. The data shows that COX-2 expression is induced sooner and to a greater extent in LPS-stimulated RAW 264.7 cells during Se deficiency. This correlates with subsequent PGE_2 production; however, Se status has no affect on COX-1 expression. This is the first report to demonstrate an inverse relationship between Se status and COX-2 expression.

MATERIALS AND METHODS

Cell culture conditions

RAW 264.7 cells, a murine macrophage cell line, were cultured at 37°C in a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM), which contained 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin (all from Life Technologies, Inc., Gaithersburg, MD, USA) and 5% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT, USA). The Se-deficient RAW 264.7 cells were grown under these conditions. The only Se present in the medium was derived from the FBS and was determined to be 6 pmoles/ml according to company quality control. The Se-supplemented RAW 264.7 cells were grown in the same medium, containing sodium selenite (Sigma, St. Louis, MO, USA) at a final concentration of 2 nmoles/ml. For the analysis of COX-2 and COX-1 expression and PGE_2 production, as well as NF-κB translocation, the cells were plated at a density of 1×10^6 cells per well in 6 well culture plates, allowed to adhere overnight, and used for these experiments the following day. Thereafter the cells were stimulated with LPS (*Escherichia coli* Serotype 0111:B4) for different time periods. The rationale for using LPS as a stimulus was to investigate some of the molecular details on how Se-deficient macrophages respond to oxidative stress as compared to Se-supplemented cells. In some experiments, N α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) (100 μM), a serine protease inhibitor of IκB degradation, was added to the cells 30 min prior to LPS challenge.

For luciferase reporter assays involving NF-κB mutants, cells were seeded at 1×10^5 cells per well in a 96 well plate and transfected with 750 ng of plasmid DNA using Superfect (Qiagen, Los Angeles, CA, USA) for 2 h. Cells were allowed to grow for 18 h prior to stimulation with LPS for 5 h. Following stimulation, cells were washed with PBS containing calcium and magnesium (1 mM each) and overlaid with 100 μl PBS. Luciferase activity was determined using LucLite Plus Assay kit (Packard, Hartford, CT, USA). The average of four transfections was used to determine the relative luciferase values for both LPS-stimulated and unstimulated cells.

Cellular Se-GPx activity assay

The cellular Se-GPx activity was measured according to the published method using H_2O_2 as substrate [19]. Selenium-deficient or Se-supplemented RAW 264.7 cells, stimulated with or without LPS (1 μg/ml), were harvested, lysed, and microfuged for 10 min. The resulting supernatant was used for enzyme analysis. The oxidation of NADPH was monitored spectrophotometrically at 340 nm following the addition of 1.5 mM H_2O_2. For comparison of individual treatments, the Se-GPx activities were expressed as nmoles of NADPH oxidized per minute per milligram of protein and the values are given as the mean of three experiments. The total protein levels were measured using the BCA reagent (Pierce, Rockford, IL, USA).

Measurement of oxidative tone

RAW 264.7 macrophage cells were grown in Se-deficient and Se-supplemented media as described in the previous section. For a sensitive measurement of total ROS generation during Se deficiency, cells were harvested, washed once with PBS, and incubated with 2.5 μM of 2′, 7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) (Molecular Probes, Eugene, OR, USA) for 15 min at 25°C. The number of cells exhibiting increased fluorescence of DCF, generated by ROS, was analyzed by a Beckman-Coulter XL-MCL single laser flow cytometer. These studies were performed at the Life Science Consortium's Flow Cytometry Facility, The Pennsylvania State University, University Park.

Isolation of nuclei

For the analysis of NF-κB translocation, nuclear extracts were prepared from Se-supplemented and deficient RAW 264.7 cells (2×10^6 cells) grown in 6 well cluster dishes. Fresh media was added to cells prior to stimulation with LPS (1 μg/ml) for the indicated times. Following stimulation, cells were washed and harvested in ice-cold PBS. Nuclear extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Pierce), according to the manufacturer's instructions. Total protein was measured using the BCA reagent (Pierce).

Western blot analyses

The harvested cells were washed three times in ice-cold PBS and centrifuged at $500 \times g$ for 10 min at 4°C. The cell pellets were resuspended in ice-cold lysis buffer (10 mM Tris pH 7.5, 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM NaPi, 10 mM NaPPi) for 15 min and then centrifuged at $10{,}000 \times g$ for 15 min. The supernatant was collected and total protein concentration was determined using the BCA reagent (Pierce). Samples of 50 μg total protein or 20 μg of nuclear protein, were electrophoresed on a 10% SDS-polyacrylamide gel and transblotted to nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) and blocked for 1 h at 25°C in 10 mM Tris, pH 8.0, containing 150 mM NaCl, 0.1% Tween 20, 5% skim milk. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. Detection of COX-2 and GAPDH or COX-1 and GAPDH were performed after incubation with their specific antibodies overnight at 4°C, while NF-κB was detected after 1 h incubation with specific antibody to p65 at 25°C. Rabbit anti-mouse COX-2 polyclonal antibody and anti-mouse COX-1 monoclonal antibody were from Cayman Chemicals (Ann Harbor, MI, USA). NF-κB p65 specific antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and monoclonal antibody for mouse anti-(GAPDH) was from Chemicon International Inc. (Temecula, CA, USA). A horseradish peroxidase-conjugated anti-IgG antibody was used as the secondary antibody. The bands were visualized by enhanced chemiluminescence (ECL) assay kit (Pierce) according to the manufacturer's instructions.

Measurements of PGE_2

Aliquots (100 μl) of culture medium were collected from the same cells grown and stimulated with LPS under identical conditions as described for Western blot analysis. PGE_2 was measured using the PGE_2 enzyme immunoassay kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instruction.

Generation of NF-κB mutants

The wild-type COX-2 promoter DNA was PCR amplified from genomic DNA isolated from RAW 264.7 cells. The promoter sequence amplified was from -966 to $+8$ with respect to the RNA start site. The wild-type promoter was cloned into pGL3 promoter vector (Promega, Madison, WI, USA) at the Bgl II and Nco I sites (which removes the SV40 promoter sequence). The NF-κB binding site at position -668 to -659 was changed from GGGAAATACC to TCGATATGAC to generate NF-κBM-666. The NF-κB binding site at position -401 to -392 was changed from GGGGATTCCC to GGTGTGTATC to generate NF-κBM-400, identical to a previously described alteration [20]. Both mutations were generated using the GeneEditor Site-directed mutagenesis system (Promega), and verified by restriction enzyme as well as sequence analyses. A double mutant construct was generated by restriction digestion of NF-κBM-666 with Bgl II and Sac I, while NF-κBM-400 was digested with Sac I and Nco I. The purified DNA fragments were sub cloned into pGL3 promoter vector digested with Bgl II and Nco I.

Electrophoretic mobility shift assay (EMSA)

Nuclear proteins (3 μg) were incubated with 15,000 cpm ^{32}P-labeled NF-κB double-stranded oligonucleotide (Promega), 1 μg poly (dI-dC), and 1.75 pmoles of unlabeled AP-1 double stranded oligonucleotide (Promega), as a nonspecific competitor, for 30 min and subjected to polyacrylamide gel electrophoresis. To confirm the specificity of NF-κB binding, unlabeled oligonucleotide (3.5 pmoles) was used as a specific competitor (SC).

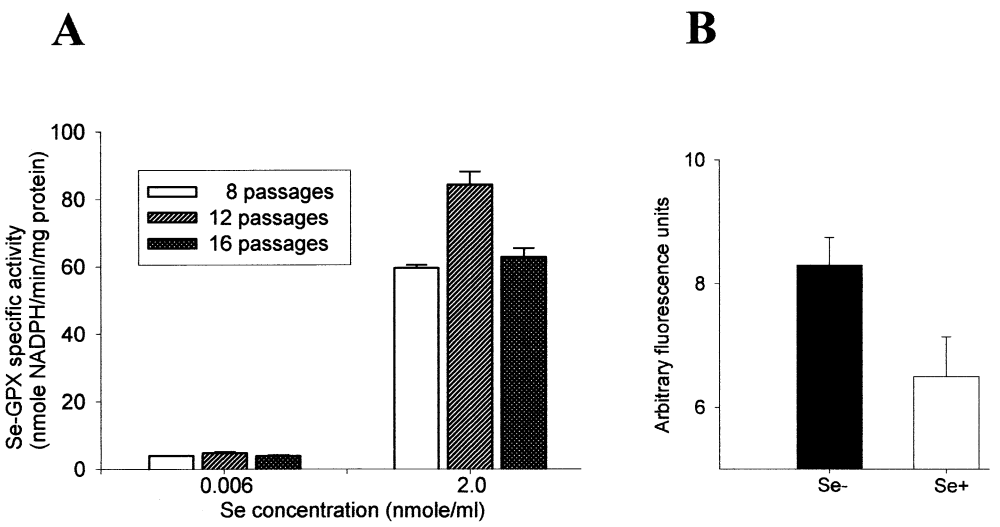

Fig. 1. Effect of Se status on Se-GPx activity and overall oxidative stress. (A) Se-GPx activities were measured at 8, 12, and 16 passages in Se-deficient or Se-supplemented (2 nmoles/ml sodium selenite) RAW 264.7 cells. The results are expressed as nmole NADPH/min/mg protein and are the mean of three independent experiments with standard error given. (B) Se-deficient and Se-supplemented RAW 264.7 cells were incubated with 2.5 μM of 2′, 7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) for 15 min. Cellular fluorescence, an indication of overall oxidative stress, was measured by a Beckman Coulter flow cytometer. Arbitrary fluorescence units for each sample, representative of three independent experiments, are given.

RESULTS

Cellular Se-GPx activity differentiates the Se-deficient and Se-supplemented RAW 264.7 cells

The cellular Se-GPx activity was measured in cell lysates from Se-deficient and Se-supplemented RAW 264.7 cells as a marker for Se status. The Se-GPx activity was determined to be significantly higher (> 10-fold) in Se-supplemented cells than that found in Se-deficient cells (Fig. 1A). The Se-GPx activity was analyzed at 8, 12, and 16 passages after initial Se-supplementation as well as after 6 months. The analyses confirm the differences between the Se-deficient and Se-supplemented cells were stable for 6 months (6 month data not shown). Selenium-dependent GPx activity was also analyzed in the cells treated with LPS in both experimental cell lines, and the results indicated no change in GPx activity due to LPS treatment (data not shown). Furthermore, incubation of RAW 264.7 cells with 2.5 μM of 2′, 7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA), a fluorescent label for oxidative tone, clearly showed that Se-deficient RAW 264.7 cells were indeed under oxidative stress even without having to be stimulated with LPS (Fig. 1B). Collectively, these data indicate that the cells have been affected in terms of oxidative tone by the Se status within the culture conditions and prior to LPS stimulation.

Selenium deficiency selectively affects the regulation of COX-2 protein expression in LPS-stimulated RAW 264.7 cells

To determine the effect of Se deficiency on the expression of COX isozymes, both Se-deficient and Se-supplemented cells were activated with LPS, and protein levels of COX-1 and COX-2 were analyzed by Western blots. Cyclooxygenase-2 protein levels were shown to increase faster and to a higher degree in Se-deficient cells during the first 4 h as compared to Se-supplemented cells (Fig. 2A and B). In contrast, COX-1 protein levels remained unaffected by either LPS stimulation or Se status during the experimental time periods. The GAPDH signal, used as internal standard, was also detected to be at the same level for all samples.

Selenium status affects PGE_2 production in LPS stimulated RAW 264.7 cells

To demonstrate that the different levels of COX-2 protein expression seen by Western blot analysis affected the overall COX-2 activity, PGE_2 production, an indicator of COX-2 activity, was analyzed by EIA. Cells were stimulated with 1 μg/ml LPS and aliquots of the cell culture supernatant were taken at various time points. Analysis of PGE_2 production demonstrated an increase in PGE_2 levels in stimulated Se-deficient cells as compared to Se-supplemented cells (Fig. 2C). The increase in PGE_2 was shown to correlate with the increase in COX-2 protein level; however, with a delayed time phase.

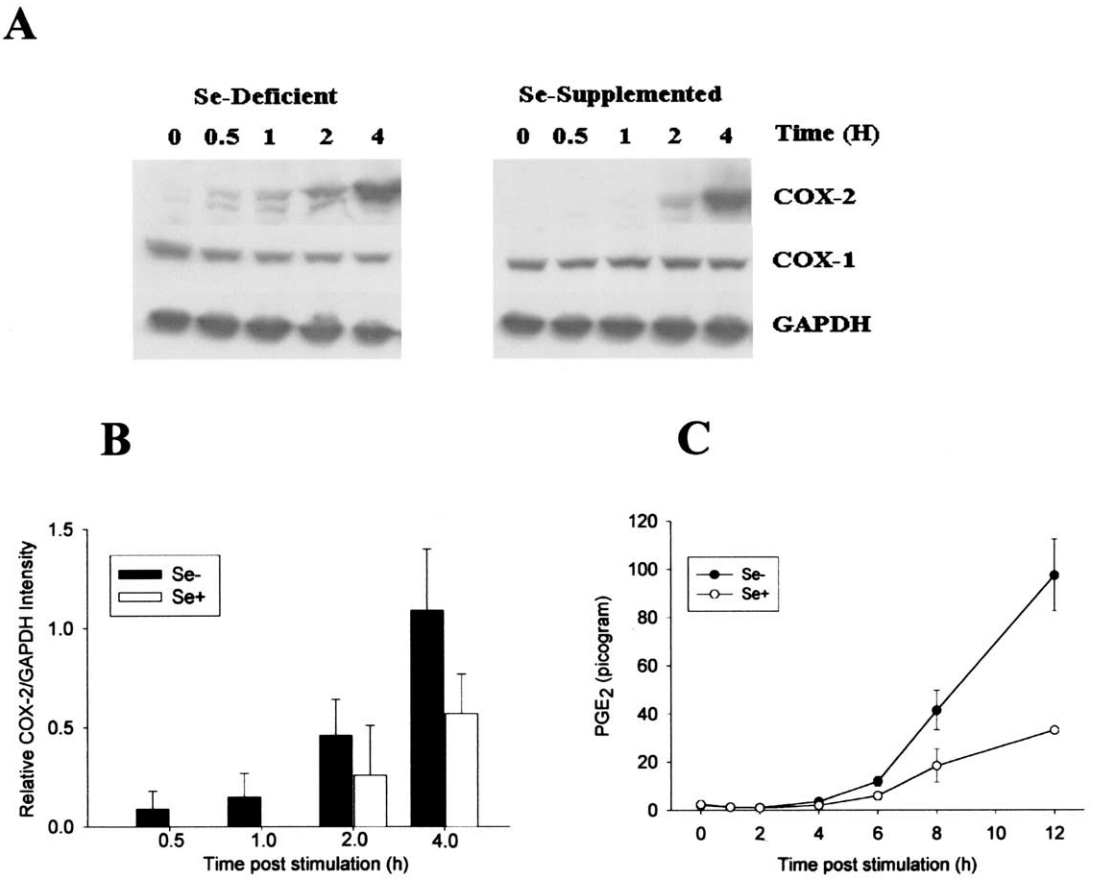

Fig. 2. Effect of Se deficiency on COX-2 protein expression and PGE_2 levels. (A) The time course of COX expression in Se-deficient and Se-supplemented RAW 264.7 cells. Cells were incubated with 1 μg/ml LPS for the indicated time periods. Cell lysates were analyzed by Western blot with the specific antibodies. The data is a representative of four independent experiments. (B) The densitometric value of the COX-2 and GAPDH specific bands from four independent Western blots was determined using Scion Image Software. The COX-2 to GAPDH ratio was determined and plotted with standard error. (C) The effect of Se status on PGE_2 production in LPS-stimulated Se-deficient and Se-supplemented RAW 264.7 cells. Cells were stimulated with 1 μg/ml LPS for the indicated time intervals (0, 2, 4, 6, and 12 h) and supernatants of the cell culture media were collected and assayed by EIA.

Cyclooxygenase-2 expression is dependent upon NF-κB activation

Recently, Wadleigh et al. reported that transcriptional activation of COX-2 in LPS stimulated macrophages was independent of NF-κB sites but rather required C/EBPβ sites [20]. Others have reported that activation of COX-2 involves NF-κB activation [21]. To determine the role of this redox-sensitive transcription factor in LPS activation of COX-2, we generated a COX-2/luciferase construct using the murine COX-2 promoter sequence from RAW 264.7 cells and then mutated the two NF-κB binding sites contained within. These constructs were transfected into RAW 264.7 cells. Following stimulation with LPS, the luciferase activity was determined. The wild type COX-2 promoter was stimulated over 2-fold with LPS when compared to the unstimulated cells (Fig. 3). Almost all the basal activity was lost when the NF-κB sites were mutated. Furthermore, the double mutant showed

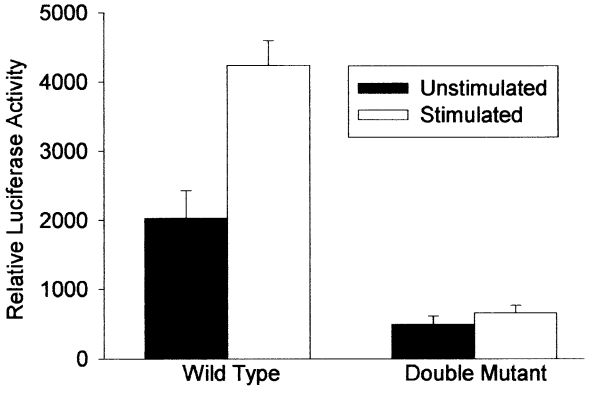

Fig. 3. LPS activation of COX-2 involves NF-κB. Both the wild-type and the double mutant promoter/reporter constructs were transfected into RAW 264.7 cells. After 18 h, one set of transfected cells was stimulated with LPS (1 μg/ml) for 5 h. Cells were washed and the luciferase activity determined. The result of four independent transfections is given with the standard error.

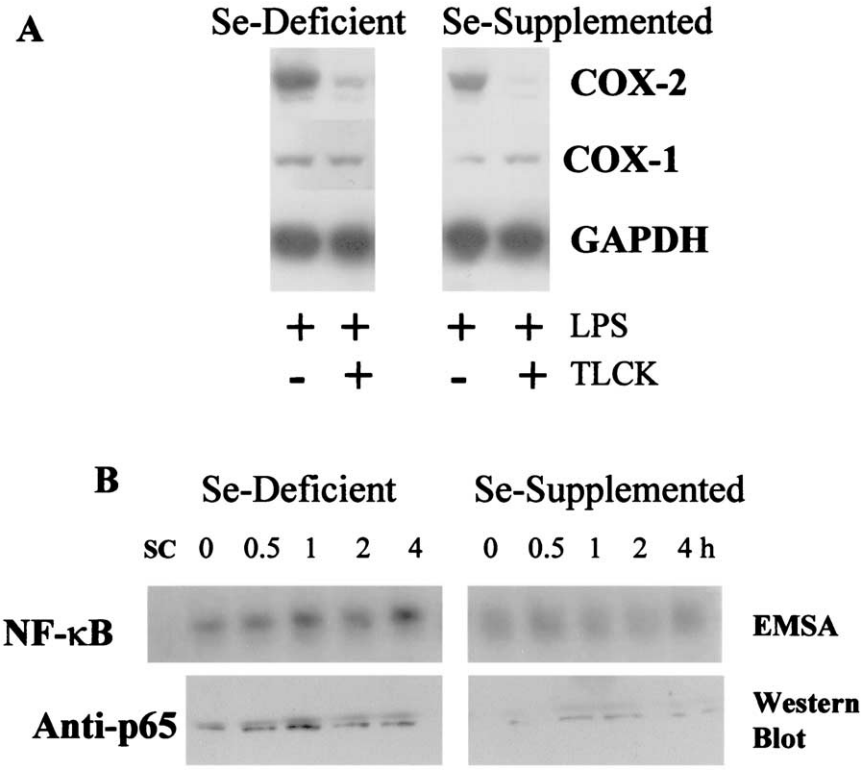

Fig. 4. NF-κB involvement in COX-2 over-expression during Se deficiency. (A) Se-deficient or Se-supplemented RAW 264.7 cells were stimulated with LPS (1 μg/ml) with or without TLCK (100 μM) for 2 h. Cell lysates were analyzed by Western blot using the indicated antibodies. (B) Se-deficient or Se-supplemented RAW 264.7 cells were stimulated with LPS (1 μg/ml) for increasing amounts of time. Cells were harvested at the indicated time and nuclei isolated. Nuclear extracts were analyzed by electrophoretic mobility shift (EMSA) (upper panel) with a ^{32}P-radiolabeled consensus κB enhancer oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′) as well as Western blot for the presence of NF-κB (lower panel). Resolved gels and blotting membranes were stained to confirm equal protein loading and uniform transfer. SC = specific competitor.

little activation with LPS. This data indicates that LPS activation of COX-2 is mediated through NF-κB.

Effects of Se status on NF-κB activation

To gain insight into the potential mechanism by which Se status affects COX-2 expression, we investigated the activation of NF-κB. TLCK is a serine protease inhibitor that has been shown to block NF-κB activation [22]. Incubation of RAW 264.7 cells with 100 μM TLCK prior to LPS stimulation blocked COX-2 expression regardless of Se status (Fig. 4A). The presence of TLCK inhibited COX-2 protein expression to basal levels suggesting that a TLCK-sensitive factor (i.e., NF-κB) is involved in the LPS activation of COX-2 expression. To further investigate the role of NF-κB, Se-supplemented and Se-deficient RAW 264.7 cells were stimulated with 1 μg/ml LPS. Nuclei were isolated from cells at various times post stimulation and nuclear extracts were analyzed by EMSA and Western blot using a NF-κB p65 specific antibody (Fig. 4B). These results indicate a significant increase in nuclear translocation of NF-κB (p65) in Se-deficient cells that is sustained longer than in the Se-supplemented cells following stimulation with LPS.

DISCUSSION

In the present study, we provide experimental support for the general hypothesis that Se, as an integral part of seleno-enzymes, may exert its anti-inflammatory and chemopreventive effect in part by inhibiting the expression of COX-2, which has been widely implicated in inflammation and tumorigenesis of many organs. Here we have demonstrated that the cellular Se status has a selective regulatory effect on COX-2 induction and subsequent PGE_2 production, without affecting COX-1 expression. The data was generated using an established RAW 264.7 macrophage model with cells grown in media deficient in Se or supplemented with Se. A concentration of 2 nmoles/ml (0.3 μg/ml) sodium selenite was found to influence the activity of selenoproteins (such as GPx) without affecting cell viability and growth rate of RAW264.7 macrophages. Cellular GPx is a functional parameter commonly used for the assessment of

Se status [2]. In our study, a 10-fold decrease in Se-GPx activity was observed in Se-deficient cells when compared to Se-supplemented cells, along with a significant increase in overall oxidant stress levels in the Se-deficient cells.

Our data suggests that cellular Se-GPX activity plays an important role in COX-2 expression. A significant increase in COX-2 protein induction was observed in RAW 264.7 macrophage cells grown in Se-deficient media as compared to cells cultured in Se-supplemented medium upon LPS stimulation, especially at early time points. In parallel to the results with COX-2 expression, the differences in PGE_2 production between Se-deficient and Se-supplemented cells increased with time post stimulation. The observed lag phase in PGE_2 formation as compared to COX-2 protein expression is most likely due, in part, to the time delay in the release of arachidonic acid necessary for the prostaglandin synthesis. The above results clearly suggest that Se status affects the regulation of COX-2 protein expression, most likely through influencing the signaling pathways to COX-2 induction.

Reactive oxygen species are important for macrophage functions such as phagocytosis and subsequent degradation of phagocytosed material; however, accumulation of excessive ROS will result in oxidative stress leading to pathophysiological events [23]. Normally, macrophages employ multiple defense strategies in tightly controlling the intracellular redox balance. For example, selenoproteins, particularly Se-GPxs and TrxRs, are important members of this antioxidant defense system [2]. Therefore, an oxidant-antioxidant imbalance, a potential consequence of inadequate Se nutrition, in macrophages can lead to important cellular changes derived, in part, from a modification of gene expression. This modification relies on transcriptional factors whose normal activities are modulated by the cellular redox environment. Among these transcriptional factors, NF-κB plays a pivotal role in inducing genes involved in the control of the immune system as well as in the response to injury and infection [15]. In addition, there is ample evidence to suggest that NF-κB is involved in the regulation of COX-2 induction by stimuli such as LPS [14]. The use of the NF-κB double mutant in the present study definitively demonstrates that COX-2 induction by LPS involves this redox-sensitive transcription factor, which is in contrast to that reported by Wadleigh et al. [20]. However, they did not mutate both NF-κB sites contained within the promoter sequence, which may explain why it was reported that NF-κB was not necessary. One point of interest is that both data show only a 2-fold increase in reporter activity in LPS-stimulated wild-type construct [20]. Since the COX-2 protein expression following LPS stimulation is greater than 2-fold, it is conceivable that sequences not contained within the reporter construct influence overall expression levels or that the luciferase expression is not as efficient as the expression of COX-2.

Our data using TLCK to inhibit NF-κB activation indicate that Se affects the COX-2 expression through a TLCK-sensitive pathway like NF-κB, since COX-2 induction in both cell lines, Se-deficient and Se-supplemented, is blocked. While TLCK is a general serine protease inhibitor, more conclusive data can be seen in the EMSA and Western analysis of nuclear extracts from LPS-stimulated cells (Fig. 4B), which shows the enhanced presence and binding of NF-κB in Se-deficient cells. Thus, Se status affects the cellular antioxidant system, which, in turn, can alter the cellular ROS levels and the sensitivity of the cells to respond to ROS. These Se-deficiency-induced changes in ROS affect signaling molecules important for initiating and activating subsequent gene induction. In particular, the increased level of ROS as a result of Se deficiency affects NF-κB activation and subsequent COX-2 expression and PGE_2 production. Selenium-deficient cells are more sensitive to the changes in cellular redox status following LPS stimulation, which results in higher and faster COX-2 protein induction. Furthermore, Se-supplemented cells are less sensitive to the changes of cellular redox status induced by LPS, most likely due to the increased levels of selenoenzymes, like Se-GPx. This helps to explain the effect of Se as a modifier of ROS levels induced by inflammation as well as Se's chemopreventive effects.

In summary, our data are the first to demonstrate that Se-supplementation selectively downregulates COX-2 expression and the enzyme activity in activated RAW 264.7 cells without altering COX-1 expression. One possible mechanism is through scavenging ROS, thereby lowering potential COX-2 activity and subsequent PGE_2 production. In agreement with this hypothesis is the observation that the redox-sensitive transcription factor NF-κB is translocated to the nucleus more rapidly and to a higher degree in Se-deficient cells. These findings propose one mechanism of Se's chemopreventive effects through altering COX-2 and PGE_2 synthesis, which in turn, alters the process of carcinogenesis as PGE_2 regulates cytokine profiles and could suppress host immunity. In addition, the moderate selective effect on the inhibition of COX-2 expression may give new insights for the design of a new family of selective COX-2 inhibitors. Collectively, such information provides additional evidence for dietary Se supplementation in the prevention and/or treatment of inflammatory diseases or cancer. The underlying molecular mechanisms, particularly the upstream protein kinases specifically responsible for NF-κB activation during Se deficiency, are currently under investigation.

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ABBREVIATIONS

COX—cyclooxygenase
LPS—lipopolysaccharide
NF-κB—nuclear factor-kappa B
PGE_2—prostaglandin E_2
Se—selenium
TLCK—N α-*p*-tosyl-L-lysine chloromethyl ketone