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# The Anti-inflammatory Effects of Selenium Are Mediated through 15-Deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ in Macrophages*

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**Selenium is an essential micronutrient that suppresses the redox-sensitive transcription factor NF-κB-dependent pro-inflammatory gene expression. To understand the molecular mechanisms underlying the anti-inflammatory property of selenium, we examined the activity of a key kinase of the NF-κB cascade, IκB-kinase β (IKKβ) subunit, as a function of cellular selenium status in murine primary bone marrow-derived macrophages and RAW264.7 macrophage-like cell line. *In vitro* kinase assays revealed that selenium supplementation decreased the activity of IKKβ in lipopolysaccharide (LPS)-treated macrophages. Stimulation by LPS of selenium-supplemented macrophages resulted in a time-dependent increase in 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15d-$PGJ_2$) formation, an endogenous inhibitor of IKKβ activity. Further analysis revealed that inhibition of IKKβ activity in selenium-supplemented cells correlated with the Michael addition product of 15d-$PGJ_2$ with Cys-179 of IKKβ, while the formation of such an adduct was significantly decreased in the selenium-deficient macrophages. In addition, anti-inflammatory activities of selenium were also mediated by the 15d-$PGJ_2$-dependent activation of the peroxisome proliferator-activated nuclear receptor-γ in macrophages. Experiments using specific cyclooxygenase (COX) inhibitors and genetic knockdown approaches indicated that COX-1, and not the COX-2 pathway, was responsible for the increased synthesis of 15d-$PGJ_2$ in selenium-supplemented macrophages. Taken together, our results suggest that selenium supplementation increases the production of 15d-$PGJ_2$ as an adaptive response to protect cells against oxidative stress-induced pro-inflammatory gene expression. More specifically, modification of protein thiols by 15d-$PGJ_2$ represents a previously undescribed code for redox regulation of gene expression by selenium.**

Macrophages play central roles as effector cells in inflammatory reactions and cell-mediated immune responses. While performing these functions, these cells produce such reactive oxygen species in the form of superoxide anion, hydrogen peroxide, hydroxyl, and lipid peroxyl radicals along with a great number of pro-inflammatory substances, including complement components, PGs,[3] chemokines, and cytokines like interleukin-1β and tumor necrosis factor-α (1). Such reactions represent a potentially toxic insult, which if not counteracted, will lead to membrane dysfunction, DNA damage and inactivation of proteins, leading to the onset and/or progression of many disease pathologies (2–4). In contrast to the conventional dogma that reactive oxygen species are mostly triggers for oxidative damage of biological structures, it is now increasingly clear that physiologically relevant concentrations of reactive oxygen species can regulate a variety of key molecular pathways that may be linked with important cell functions, including gene expression (5). Regulation of intracellular reactive oxygen species levels is accomplished by a complex multitier antioxidant system, which plays an important regulatory role in the expression of pro-inflammatory genes (6). The antioxidant capacity of cells, including macrophages, is therefore, very important not only to maintain its own vital function in its defense but also to tightly control the intracellular oxidative tone. In the multitier antioxidant system, micronutrient selenium plays a major role mainly in the form of selenoproteins (7). Thioredoxin reductases and glutathione peroxidases (GPXs) represent two well studied classes of selenoenzymes that catalyze the reduction of cellular peroxides and help in maintaining an optimal redox balance in cells (8). The activity of selenoenzymes is directly proportional to the total plasma selenium (9). However, in many disease states, including HIV

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[3] The abbreviations used are: PG, prostaglandin; GPX, glutathione peroxidase; HIV, human immunodeficiency virus; NF-κB, nuclear factor-κB; COX, cyclooxygenase; IKKβ, IκB-kinase β; 15d-$PGJ_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$; PPAR, peroxisome proliferator-activated receptor; H-PGDS, hematopoietic $PGD_2$ synthase; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; BMDM, bone marrow-derived macrophage; GST, glutathione *S*-transferase; GW9662, 2-chloro-5-nitro-*N*-phenylbenzamide; HQL-79, 4-(diphenylmethoxy)-1-[3-(1H-tetrazol-5-yl)propylpiperidine]; LC-MS, liquid chromatography-mass spectrometry; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.

and many cancers, the activity of selenoenzymes is reduced leading to inflammation and decreased immune function (10). In recent years, the role of selenium in preventing human disease has gained new attention following the association of "super supplementation" with decreased incidences of prostate cancer in a few preliminary studies (11–13). It has been proposed that selenium prevents malignant transformation of cells by serving as a "redox switch" through its role in catalyzing oxidation-reduction reactions of critical thiol groups or disulfide bonds, possibly through selenoproteins (14, 15).

Recent studies have shown selenium to be a potent regulator of transcription pathways. In fact, selenium deficiency in human Jurkat and lung carcinoma cells increased the nuclear binding and transcriptional activation of oxidative-stress responsive genes by nuclear factor-κB (NF-κB) (16). Along the same lines, studies in our laboratory have shown that selenium supplementation of macrophages decreased the expression of two pro-inflammatory genes, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase, via the inactivation of NF-κB, whereas the expression of COX-1 was unaffected (17, 18). It is well known that signaling pathways activated by ligation of cell surface pattern recognition receptors converge at the IκB kinase (IKK) complex that phosphorylates the inhibitory subunit and cytoplasmic retention protein of the NF-κB complex, IκBα (19). The IKK complex includes highly homologous catalytic kinases, IKKα (IKK1), IKKβ (IKK2), and an essential regulatory subunit, also known as IKKγ or NEMO (20). Although both IKKα and IKKβ can phosphorylate all three IκB proteins *in vitro*, studies in mice that are deficient in IKK subunits show that, in most cells, IKKβ has the dominant role in signal-induced phosphorylation and degradation of these proteins (21, 22). In addition to phosphorylation by upstream kinases, the enzymatic activity of IKKβ is subjected to further control by Michael adduct formation with α,β-unsaturated carbonyl compounds, 4-hydroxynonenal (23) or cyclopentenone PGs such as $PGA_1$ and 15d-$PGJ_2$, with a critical cysteine (Cys-179) residue in the activation loop (24). Thus, the inhibition of NF-κB activation, by these endogenous compounds, could play a seminal role in the resolution of immune response. In addition, 15d-$PGJ_2$ has been shown to directly bind and activate the nuclear hormone receptor PPARγ (25), which can trans-repress the inflammatory responses mediated by NF-κB (26–28). Therefore, cellular production of 15d-$PGJ_2$ may mediate anti-inflammatory responses via these pathways. However, there are no reports on the preferential increase in the intracellular levels of 15d-$PGJ_2$ to support these claims. Here, we show for the first time that selenium supplementation of macrophages leads to the preferential increase in 15d-$PGJ_2$ via the arachidonic acid oxidation by COX-1, rather than COX-2. In macrophages and other immune cells, $PGH_2$ is further converted to $PGD_2$ by the hematopoietic $PGD_2$ synthase (H-PGDS) that undergoes two spontaneous non-enzymatic dehydration reactions to form 15d-$PGJ_2$ (29). The present study is based on the hypothesis that the anti-inflammatory role of selenium occurs, in part, via 15d-$PGJ_2$-dependent intracellular signaling pathways in macrophages and that COX-1 plays a pivotal role in the control of NF-κB activity.

## MATERIALS AND METHODS

*Reagents*—Bacterial endotoxin lipopolysaccharide (LPS), sodium selenite, and GW9662 were from Sigma. Anti-COX-1, anti-COX-2, COX inhibitors, indomethacin, SC-560 (for COX-1), and CAY10404 (for COX-2) were obtained from Cayman Chemicals (Ann Arbor, MI). Antibodies for IKKα, pIκBα, glyceraldehyde-3-phosphate dehydrogenase, and p65 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-IKKβ was from Imgenex (San Diego, CA). Anti-15d-$PGJ_2$ was from Assay Designs (Ann Arbor, MI). Goat anti-rabbit IgG, anti-mouse IgG conjugated to horseradish peroxidase, polyvinylidene difluoride, and West Pico chemiluminescence reagents were purchased from Pierce. Mouse IKKβ-(173–186) was synthesized at the Macromolecular core facility, Pennsylvania State College of Medicine, Hershey, PA.

*Cell Culture and Stimulation*—The murine macrophage cell line RAW264.7 (ATCC) was cultured in Dulbecco's modified Eagle's medium containing 5% defined fetal bovine serum (HyClone), 80 μg/ml gentamicin, and 2 mM L-glutamine (Invitrogen) at 37 °C with a 5% $CO_2$/air mixture. Total selenium in the fetal bovine serum was quantitated to be 6 pmol/ml. Cells were cultured in Dulbecco's modified Eagle's medium either supplemented with selenium (2 nmol/ml of media) or without any added as described from our laboratory (17). Cell viability and growth rates of selenium-supplemented cells were similar to their selenium-deficient counterparts. The enzymatic activity was used as a marker of cellular selenium status (9). About $1 \times 10^6$ selenium-deficient and selenium-supplemented cells were seeded in a 6-well plate, and then cultured in respective media for ~24 h to allow the cell number to approximately double. Cells were stimulated with LPS (0–1 μg/ml) and/or other compounds for the indicated time periods. Upon treatment, the cells were harvested, washed with cold sterile PBS and stored at −80 °C until further use. Femoral bone marrow plugs, from mice maintained on a selenium-deficient or a selenium-supplemented diet, were isolated, and adherent cells, hereafter referred to as primary bone marrow-derived macrophages (BMDMs), were differentiated in their respective media containing 20% L929 fibroblast media supernatant (as a source of granulocyte-macrophage colony-stimulating factor) for 1 week. The L929 cells were also cultured under selenium-deficient or selenium-supplemented conditions. The selenium-deficient and selenium-supplemented diets were formulated based on an American Institute of Nutrition recommended rodent diet containing 0.01 or 0.4 ppm of selenium, as described (30). The diets were purchased from Zeigler (Gardners, PA). The BMDM cultures from selenium-deficient and selenium-supplemented mice were used in all experiments.

*LPS Treatment of Mice*—Selenium-deficient and selenium-supplemented mice ($n = 3$ in each category) were treated with *Escherichia coli* serotype 0111:B4 LPS (at 5 mg/kg body weight) or PBS control by intraperitoneal injection. The mice were euthanized after 6 h of injection. The serum from each mouse was prepared and used in 15d-$PGJ_2$ enzyme-linked immunosorbent assays as described later in this section. All animal protocols were approved by the Institutional Animal Care and Use Committee.

*Preparation of Cell Lysates*—The frozen cell pellet was resuspended in 50 $\mu$l of mammalian protein extraction reagent (M-PER, Pierce) containing 1 mM EDTA, 10 $\mu$M leupeptin, and 1 mM phenylmethylsulfonic acid for 30 min on ice with intermittent vortexing. Supernatants were prepared by centrifuging the cell lysate at 10,000 × *g* for 15 min at 4 °C and used for analyses. Protein concentration in the cell supernatants was determined by BCA protein assay (Pierce).

*Electrophoresis and Immunoblotting*—Thirty micrograms of protein from RAW264.7 or BMDM cell lysates was separated on a 12.5% SDS-polyacrylamide gel and transblotted onto polyvinylidene difluoride membrane as described (31). The membrane was blocked with Tris-buffered saline containing 0.05% Tween 20 and 5% skim milk (w/v). The membrane was probed with primary antibody followed by an appropriate secondary antibody coupled to horseradish peroxidase.

*Immunoprecipitation*—Cell lysates (~30–50 $\mu$g of protein) from LPS-treated and untreated selenium-deficient or selenium-supplemented RAW264.7 and BMDMs were used with IKK$\alpha$- (Santa Cruz Biotechnology) or IKK$\beta$-specific IgG (Imgenex). The immunoprecipitated IKK·IgG complex was pulled down using Protein A/G-Sepharose (GE Amersham Biosciences) and used in Western blot analysis, as described above, or *in vitro* kinase assays, as described below. The membrane was probed with anti-15d-$PGJ_2$.

*[$^{14}$C]Arachidonic Acid Treatment of Macrophages*—The selenium-deficient and selenium-supplemented RAW264.7 cells were pretreated with [1-$^{14}$C]arachidonic acid (1 $\mu$Ci and 30 $\mu$M, American Radiochemicals, St. Louis, MO) for 2 h prior to stimulation with LPS for 1 h. The cell lysates were used to isolate IKK$\beta$ by immunoprecipitation, as described earlier, and analyzed on an SDS-PAGE followed by autoradiography. The blots were reprobed for IKK$\beta$ to confirm near-equal immunoprecipitation.

*In Vitro Kinase Assays*—Cell lysates for *in vitro* kinase assays were prepared using the lysis buffer as described (32). In the case of IKK, the cell lysate (~30–50 $\mu$g of protein) was incubated with GST-I$\kappa$B$\alpha$ and ATP for 1 h in the kinase wash buffer (50 mM Tris-Cl, pH 8.0, containing 100 $\mu$M NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 10 mM $\beta$-glycerophosphate, 10 mM NaF, and 1 mM sodium vanadate) at 25 °C. Glutathione-Sepharose beads (Amersham Biosciences) were added to the reaction mixture, and the mixture was centrifuged. The pellet was washed three times with sterile PBS. The phospho-labeled GST-I$\kappa$B$\alpha$ was separated by SDS-PAGE and immunoblotted onto polyvinylidene difluoride membranes. The membrane was probed with anti-phosphoserine (Sigma). Furthermore, to quantitate the activity, immunoprecipitated IKK$\alpha$ or IKK$\beta$ was incubated with [$^{32}$P]ATP (2 $\mu$Ci, Amersham Biosciences) and GST-I$\kappa$B$\alpha$ fusion protein as described (32). The reaction mixture was subjected to gel-filtration chromatography using the Bio-Gel P30 pre-packed columns (Bio-Rad). The flow-through, which contained the GST-[$^{32}$P]I$\kappa$B$\alpha$, was subjected to liquid scintillation counting.

*Chemical Treatments*—Selenium-deficient and selenium-supplemented RAW264.7 and BMDM cells ($1 \times 10^6$) were seeded into each well of a 6-well plate and treated with 1 $\mu$M indomethacin or SC-560 to inhibit COX-1. 10 nM CAY10404 was used as a COX-2-specific inhibitor. In all cases, the treatment with inhibitors (for 24 h) was followed by stimulation with LPS for the indicated time periods. The cell lysates were prepared as described earlier and subjected to Western immunoblot analyses, *in vitro* kinase assays, while the media supernatants were used for 15d-$PGJ_2$ assays. An irreversible PPAR$\gamma$ antagonist, 2-chloro-5-nitro-*N*-phenylbenzamide (GW9662, 1 $\mu$M) was used in some studies to determine the specific role of 15d-$PGJ_2$-dependent activation of PPAR$\gamma$. The cells were pretreated with GW9662 for 12 h prior to LPS stimulation. To inhibit the activity of H-PGDS, selenium-supplemented cells were pretreated with 50 $\mu$M 4-(diphenylmethoxy)-1-[3-(1H-tetrazol-5-yl)propylpiperidine] (HQL-79, Cayman), prior to LPS stimulation for 12 h. Supernatants were used in 15d-$PGJ_2$ analysis as described below. All chemical treatment studies included an appropriate $Me_2SO_4$ vehicle control.

*Quantitation of $PGD_2$ and 15d-$PGJ_2$*—A 96-well-based enzyme immunoassay kit, from both Cayman Chemicals and Assay Designs (Ann Arbor, MI), was used to quantitate $PGD_2$ and 15d-$PGJ_2$, respectively. The concentrations of 15d-$PGJ_2$ in culture media supernatants of RAW264.7 and BMDM in the presence or absence of LPS (0–1 $\mu$g/ml) for various time periods were determined by enzyme immunoassay according to the manufacturer's instructions and normalized to total cellular protein. $PGD_2$ was derivatized using methoximylamine-HCl as per the recommendation of the supplier. Standard calibration curves were prepared using $PGD_2$ methoxime or 15d-$PGJ_2$ and fitted to a log-linear, logit, multiway frequency regression analysis. $PGD_2$ and 15d-$PGJ_2$ were quantitated in cell lysates and normalized to total protein in the cell lysates.

*LC-MS Analysis of 15d-$PGJ_2$ Production*—Culture media supernatants from LPS-stimulated selenium-deficient and selenium-supplemented macrophages were acidified with 2 N HCl and clarified by centrifugation at 10,000 × *g* for 5 min. Supernatants were processed using a C18-Sep-Pak column cartridge (Waters), and bound 15d-$PGJ_2$ was eluted with methanol, evaporated, and stored in ethyl acetate at −80 °C until further analysis. As an internal control to calculate extraction efficiency, 200 ng of deuterated 15d-$PGJ_2$(d4) (Cayman Chemicals) was added to the supernatants before extraction. The 15d-$PGJ_2$ was resolved on a Restek Ultra Aqueous C18 (5 $\mu$m, 250 mm × 2.1 mm) high-performance liquid chromatography column (Bellefonte, PA) on an aqueous acetonitrile gradient with 0.1% formic acid at a flow rate of 0.2 ml/min. MS analysis was performed on a Micromass ZMD mass spectrometer (Waters) set to scan mode ($m/z$ 200–400) for authentic standards, whereas selective ion monitoring set to $m/z$ 317 (M-$H^+$) and 321 (M-$H^+$) for 15d-$PGJ_2$(d0) and 15d-$PGJ_2$(d4), respectively, was used for quantitation in the samples. Standard calibration curves for 15d-$PGJ_2$(d0) and 15d-$PGJ_2$(d4) were set up for the quantitation and calculations were performed based on the following: $b(0) = 6545.09$, $b(1) = 25.51$, and $r^2 = 0.99$ (for d0) and $b(0) = 253.48$, $b(1) = 79.646$, and $r^2 = 0.98$ (for d4).

*Mass Spectrometric Analysis of Post-translational Modification of IKK$\beta$*—To further confirm the modification of IKK$\beta$ by 15d-$PGJ_2$ and support the immunoprecipitation studies, murine IKK$\beta$ peptide-(173–186), LDQGSL<u>C</u>TSFVGTL, was incubated with $Me_2SO$, authentic 15d-$PGJ_2$ (mol/mol), or total lipid extract from selenium-supplemented macrophages (LPS-

treated for 2 h) for 30 min at 37 °C in PBS. The samples were analyzed by MALDI-TOF-MS for modification.

*Transient Transfection Assays*—Murine COX-2 gene promoter (−2000 to +75) and its NF-κB double mutant in $pGL_3$ luciferase vector were prepared as described (17). The plasmid constructs were transfected into RAW264.7 cells using FuGENE 6 transfection reagent as per the instructions of the supplier (Roche Applied Science). To normalize the transfection efficiency, β-galactosidase activity from the pSV-βGal (Promega, Madison, WI) plasmid and total protein were used. RAW264.7 cells were stimulated with LPS for 4 h post-transfection. Cell lysates were prepared as described above, and the luciferase activity was read in a Turner plate luminometer.

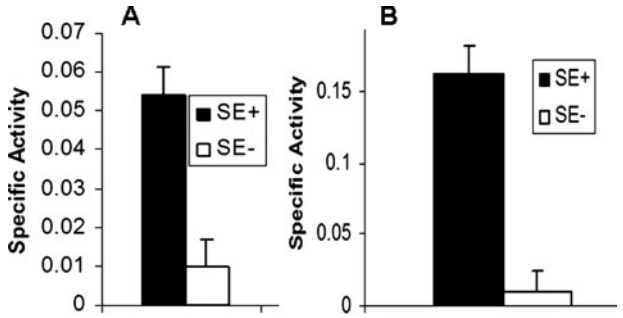


FIGURE 1. **Cytosolic GPX activity in BMDM (*A*) and RAW264.7 (*B*) macrophages.** Primary bone marrow-derived macrophages and RAW264.7 macrophages were cultured in the presence or absence of selenium for ~1 week. Cell extracts were used in GPX assays with $H_2O_2$ as substrate. The specific activity is described as the amount of protein required to oxidize 1 μmol of NADPH per minute. Representative of $n = 3$ experiments.

*siRNA Experiments*—The siRNA target sequence for murine COX-1 mRNA was designed using the Dharmacon siGENOME™ design tool available online. The 21-base siRNA oligonucleotides were obtained from Dharmacon and annealed according to the manufacturer's specifications. For transfection, RAW264.7 or BMDM was seeded into 6-well plates ($1 \times 10^6$ cells/well) without gentamicin. After 24 h, cells were transfected with siRNA duplex using TransIT®-siQUEST™ (Mirus Bio Corp.) according to the manufacturer's specifications. siRNA duplexes were used at a final concentration of 200 pmol/well. To ensure maximum effect, a second transfection was performed after 4 h, and the cells were allowed to recover for an additional 8 h before treatment with LPS. Cells and media supernatants from LPS-treated (12 h) or untreated cells were collected for 15d-$PGJ_2$ quantitation, whereas the corresponding cell lysates were used for IKKβ activity and Western immunoblot analyses.

*Preparation of Nuclear Extracts for Electrophoretic Mobility Shift Assay*—For electrophoretic mobility shift assay experiments, nuclear proteins were isolated as described previously from our laboratory (31). The DNA sequences of the sense strand of double-stranded oligonucleotides specific for NF-κB and PPARγ were 5′-GATCCAGTTGAGGGGACTTTCCCAGGC-3′ and 5′-GGTGAGGAGGGGAAGGGTCAGTGTG-3′, respectively. Complementary strands were annealed, and double-stranded oligonucleotides were labeled with [γ-$^{32}$P]ATP (3000 Ci/mmol and 10 mCi/ml) using the T4 polynucleotide kinase (New England Biolabs). Five micrograms of nuclear proteins was incubated for 10 min at 4 °C in a binding buffer specific for NF-κB (20 mM Tris-HCl, pH 7.9, 5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, and 20% glycerol) or PPARγ (20 mM HEPES, pH 7.5, 50 mM KCl, 0.175 mM EDTA, and 5% (v/v) glycerol) in the presence of 2 μg of poly(dI-dC). The extracts were then incubated for 30 min at 4 °C with 10,000 cpm of $^{32}$P-labeled NF-κB or PPARγ probes. The samples were analyzed as described earlier from our laboratory. NF-κB or PPARγ bands were confirmed by competition with a 100-fold excess of the respective unlabeled probe.

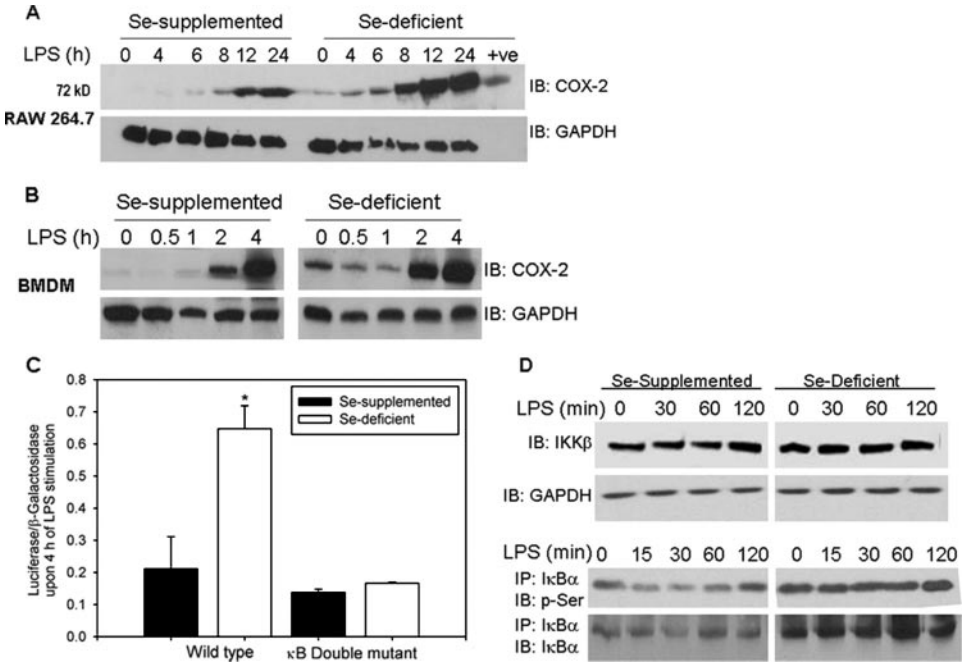


FIGURE 2. **Selenium supplementation leads to inhibition of NF-κB-dependent COX-2 expression.** *A*, selenium-supplemented and selenium-deficient RAW264.7 cells were stimulated with 1 μg/ml LPS for various time periods, and the cell lysates were analyzed for the expression of IKKβ expression, levels of pIκBα, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). *B*, BMDMs isolated from selenium-supplemented or selenium-deficient mice were stimulated with LPS for 0–4 h, and the cell lysates were used for Western immunoblot analysis for COX-2 and GAPDH expression. *C*, the murine COX-2 promoter luciferase reporter constructs of the wild-type and NF-κB double mutant were transiently transfected into RAW264.7 macrophages for 24 h followed by stimulation with LPS for 4 h. To normalize the transfection efficiency, the β-galactosidase activity from the co-transfected plasmid, pSVGal, was used. The luciferase activities of the unstimulated cells were used to calculate the -fold increase upon stimulation with LPS (*, $p < 0.05$). *D*, selenium-supplemented or selenium-deficient RAW264.7 macrophages were also stimulated for 0–2 h with LPS, and the cell lysates were used for Western immunoblot analysis of expression of IKKβ, total IκBα, and pIκBα as described above. The blots were exposed to the x-ray film for identical times. All experiments were performed in triplicates and representative in each case is shown.

## RESULTS

*Differential Selenium Status in Macrophages*—Culturing of RAW-264.7 cells in the presence or

absence of selenium, as well as primary BMDMs isolated and differentiated from mice maintained on selenium-deficient and selenium-supplemented diets, yielded cell populations that exhibited differential selenium status as seen by cytosolic GPX activity levels (Fig. 1, *A* and *B*). A 6-fold difference in the cytosolic GPX activity in the selenium-deficient and selenium-supplemented BMDM was seen. Along the same lines, liver homogenates from selenium-deficient and selenium-supplemented mice demonstrated a 7-fold difference in the enzymatic activity of cytosolic GPX (data not shown). In the case of RAW264.7 macrophages, a difference of ~12-fold was seen between the two groups (Fig. 1*B*). The results of differences in GPX activity are consistent with those previously reported in RAW264.7 macrophages from our laboratory (17). Furthermore, the expression of GPX1 were also found to be significantly different in the two groups in that the selenium-supplemented cells demonstrated a higher level of GPX1 expression compared with the selenium-deficient cells (data not shown). Accordingly, the selenium-supplemented and selenium-deficient BMDM and RAW264.7 macrophages were used in all the experiments described below.

*Selenium Deficiency Exacerbates COX-2 Expression via Increased Levels of pIκBα Leading to the Activation of NF-κB*—Stimulation of selenium-deficient RAW264.7 and BMDM cells with LPS for 0–24 h clearly demonstrated exacerbated expression of COX-2 when compared with those cultured in the presence of selenium (Fig. 2, *A* and *B*). The differences in expression were obvious as early as 30 min of stimulation of cells (Fig. 2*B*). Previous reports from our laboratory have indicated that NF-κB is a key transcription factor in the regulation of COX-2 expression in selenium-deficient cells (17). To further understand the role of selenium on the NF-κB-dependent transcription of COX-2, we mutated the two NF-κB sites in the promoter of murine COX-2. As seen in Fig. 2*C*, the transient transfection studies with wild-type mouse COX-2 promoter luciferase reporter exhibited a 4- to 5-fold increase in activity in selenium-deficient RAW264.7 cells, whereas there was no increase noted in the NF-κB double mutant reporter. The abrogation of luciferase activity upon LPS stimulation in the NF-κB double mutant clearly suggested that NF-κB plays an important role in the regulation of COX-2 expression. These results also indicated an alteration in the upstream signaling mechanisms, such as the activation of IKK family of enzymes by cellular selenium status. Although Western immunoblots showed no obvious differences in the expression of IKKβ in LPS-stimulated selenium-deficient or selenium-supplemented cells, the levels of both IκBα and pIκBα were significantly higher in the former group (Fig. 2*D*). This sustained increase in IκBα/pIκBα corroborates well with the increased translocation of p65 in selenium-deficient cells described earlier from our laboratory (17).

*Selenium Supplementation Decreases the Activity of IKKβ*—Because selenium affected the levels of cytosolic pIκBα, without altering the expression of IKKβ, we monitored the enzymatic activity of IKKβ in both RAW264.7 and BMDMs cultured in the presence or absence of selenium. Using *in vitro* kinase assays we found that, in selenium-supplemented cell lines, IKKβ activity was significantly reduced. The activity further declined upon LPS stimulation of these cells up to 2 h. On the other hand, in selenium-deficient cells, the activity of IKKβ increased with LPS stimulation (Fig. 3, *A* and *B*). In addition, *in vitro* kinase assays of immunoprecipitates containing IKKα and IKKβ from selenium-deficient and selenium-supplemented RAW264.7 cells following LPS stimulation demonstrated increased activity of IKKβ in LPS-treated selenium-deficient cells, whereas, in the selenium-supplemented cells, there was no such increase in activity upon treatment with LPS (Fig. 3*C*). On the other hand, activity of IKKα was not affected by selenium status (Fig. 3*C*).

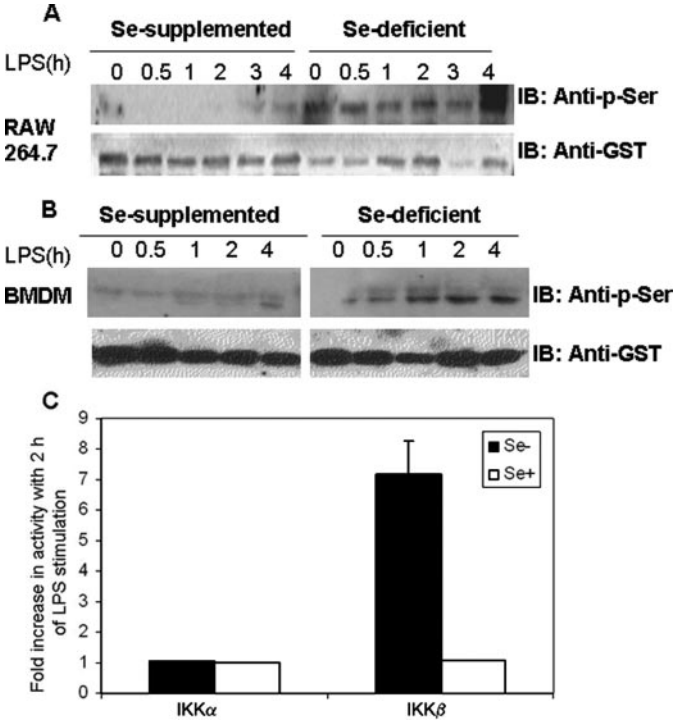


FIGURE 3. **Selenium supplementation of macrophages represses the activity of IKKβ.** *A* and *B*, total kinase activities in RAW264.7 (*A*) and BMDM cells (*B*), cultured in the presence or absence of selenium, and stimulated with LPS for the indicated time periods. Cell lysates were incubated with GST-IκBα fusion protein and subjected to GST pulldown assay with GSH-agarose beads. The phosphorylation status of the GST-IκBα was analyzed by Western immunoblot with anti-p-Ser monoclonal antibodies. *C*, RAW264.7 cell lysates (50 μg) stimulated with LPS ($t = 2$ h) were used in immunoprecipitation reactions with anti-IKKα or anti-IKKβ. The immunoprecipitates were used in *in vitro* kinase assays with [γ-$^{32}$P]ATP and GST-IκBα. The -fold increases in activity were calculated with respect to unstimulated cells. Representative of $n = 3$ experiments.

*Enhanced Production of 15d-PGJ$_2$ in Selenium-supplemented Macrophages*—The above results suggested that the enzymatic activity of IKKβ was severely affected by some mechanism almost exclusively in the selenium-supplemented cells and not in the selenium-deficient cells. Because cellular selenium status did not affect IKKβ expression (Fig. 2*D*), we hypothesized that post-translational modification of IKKβ was a likely possibility. Previous research in our laboratory has indicated that selenium-supplemented cells produced less PGE$_2$ upon LPS stimulation compared with the selenium-deficient cells (17). We reasoned that, in selenium-supplemented cells, PGH$_2$ from COX-1 or low levels of COX-2 was possibly shunted to H-PGDS to produce PGD$_2$ and subsequently converted to 15d-PGJ$_2$ to affect IKKβ activity. Surprisingly, the levels of 15d-PGJ$_2$ in the culture supernatant of selenium-supplemented BMDMs

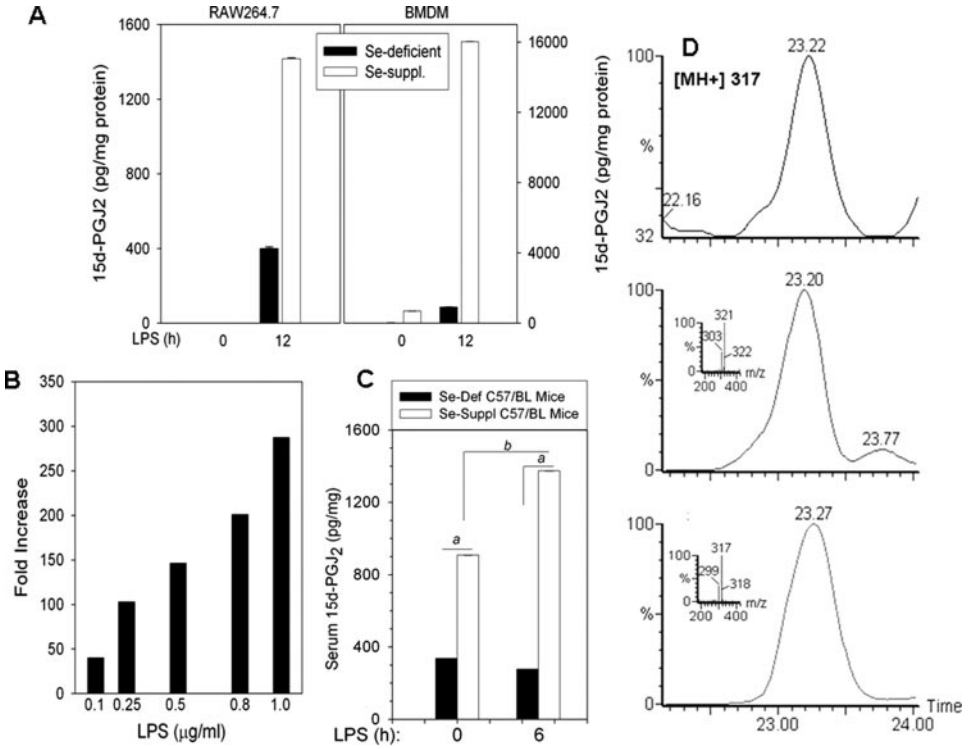


FIGURE 4. **Selenium supplementation leads to increased production of 15d-PGJ$_2$.** *A*, selenium-supplemented or selenium-deficient RAW264.7 (*left panel*) or BMDM macrophages (*right panel*) were stimulated with LPS for 0 and 12 h. The cell culture supernatants were quantitated by enzyme-linked immunosorbent assay for 15d-PGJ$_2$. The values were normalized to the total cellular protein levels ($n = 3$). *B*, dose-dependent effect of LPS on 15d-PGJ$_2$ production in BMDM. Media supernatant from selenium-supplemented BMDM stimulated with LPS (0–1 $\mu$g/ml) for 12 h was used in the quantitation of 15d-PGJ$_2$. *C*, 15d-PGJ$_2$ production in mice. Serum levels of 15d-PGJ$_2$ in selenium-deficient and selenium-supplemented mice ($n = 3$ in each category) were quantitated by enzyme-linked immunosorbent assay before and after intraperitoneal injection with LPS or PBS for 6 h. *a* and *b* represent $p < 0.005$ compared with selenium-deficient and selenium-supplemented at $t = 0$, respectively. *D*, LC-MS profiles of 15d-PGJ$_2$. *Top panel*: selective ion monitoring (M-H$^+$) at 317 *m/z* in extracellular culture media from selenium-supplemented RAW264.7 cells; *middle* and *bottom panels*: scan (200–400 *m/z*) of authentic 15d-PGJ$_2$(d4) and 15d-PGJ$_2$(d0) with the spectrum showing the fragmentation of the parent ions 321 *m/z* (M-H$^+$) and 317 *m/z* (M-H$^+$) for 15d-PGJ$_2$(d4) and 15d-PGJ$_2$(d0), respectively.

was significantly higher, prior to stimulation with LPS, when compared with those cultured in the absence of selenium (Fig. 4*A*). In RAW264.7 cells, we failed to observe any generation of 15d-PGJ$_2$ in the unstimulated cells (Fig. 4*A*). Upon LPS stimulation of BMDMs and RAW264.7 cells for 12 h, 15d-PGJ$_2$ increased only in the selenium-supplemented cells and not in the selenium-deficient group (Fig. 4*A*). Similar results were also obtained in lysates of selenium-supplemented cells treated with LPS for 2 h (data not shown). An extended time course experiment up to 24 h of LPS stimulation revealed further increases in 15d-PGJ$_2$ only in the selenium-supplemented cells (data not shown). Interestingly, the LPS-stimulated selenium-supplemented BMDMs produced ~10-fold higher 15d-PGJ$_2$ than the corresponding selenium-supplemented and LPS-stimulated RAW264.7 cells (Fig. 4*A*). A dose-dependent effect of LPS (0.1–1.0 $\mu$g/ml) on 15d-PGJ$_2$ was also seen in BMDM cells (Fig. 4*B*). To further rule out any *in vitro* effect, serum of selenium-deficient and selenium-supplemented mice with or without LPS treatment for 6 h, clearly demonstrated a similar time-dependent increase in 15d-PGJ$_2$ only in the selenium-supplemented mice (Fig. 4*C*). Supplementation of selenium-deficient RAW264.7 cells with 2 $\mu$M sodium selenite for 24 h followed by 12 h of LPS stimulation increased the production of 15d-PGJ$_2$ by 8- to 10-fold compared with the untreated cells stimulated with LPS (data not shown). The increases in 15d-PGJ$_2$ in selenium-supplemented macrophages were also confirmed by LC-MS (Fig. 4*D*). Based on this LC-MS method, the quantitation indicated that selenium-supplemented RAW264.7 cells produced 7-fold higher 15d-PGJ$_2$ compared with selenium-deficient cells upon 12 h of LPS treatment (data not shown).

*Increase in 15d-PGJ$_2$ Is Dependent on H-PGDS Activity*—To examine if a substrate-product relationship existed between PGD$_2$ and 15d-PGJ$_2$ in selenium-supplemented macrophages, we quantitated the PGD$_2$ formed in the culture media supernatant of cells as a function of time post LPS stimulation (0–12 h). Results clearly demonstrate that, within the first 6 h of LPS treatment, PGD$_2$ levels increased significantly with a peak ~6 h and a decline at ~8 h (Fig. 5*A*). In contrast, 15d-PGJ$_2$ levels began to increase after 8 h of LPS treatment. Incubation of selenium-supplemented macrophages with HQL-79, an H-PGDS inhibitor, completely inhibited the increase in 15d-PGJ$_2$ production (Fig. 5*B*).

*Formation of IKK$\beta$-15d-PGJ$_2$ Adduct in Selenium-supplemented Cells Accounts for the Decreased NF-$\kappa$B Activation and COX-2 Expression*—As a consequence of production of 15d-PGJ$_2$ in selenium-supplemented cells, an increased interaction of 15d-PGJ$_2$ with IKK$\beta$ subunit was observed in such cells, which increased upon stimulation with LPS. The formation of adduct correlated well with the increased intracellular production of 15d-PGJ$_2$ (0–2 h, data not shown) and decreased IKK$\beta$ activity in selenium-supplemented cells (Fig. 6*A*). To investigate if the IKK$\beta$ subunit was differentially modified with an endogenous product of arachidonic acid oxidation, we performed radiolabeling studies with [$^{14}$C-1C]arachidonic acid added to RAW264.7 cells for 2 h prior to stimulation with LPS for various time periods up to 1 h. As shown in Fig. 6*B*, IKK$\beta$ immunoprecipitated from selenium-supplemented cells showed increased formation of radiolabeled-adduct with LPS stimulation, whereas, relatively lower levels of adduct formation were observed in selenium-deficient cells even in the presence of LPS (Fig. 6*B*). Furthermore, the interaction of IKK$\beta$ with 15d-PGJ$_2$ in the selenium-supplemented cell lysates from LPS (2 h) was confirmed using a murine IKK$\beta$-(173–186)-peptide in qualitative MALDI-TOF-MS assays. The results clearly demonstrated an increase in the mass of the peptide from 1440.7 to 1757.2, a difference of

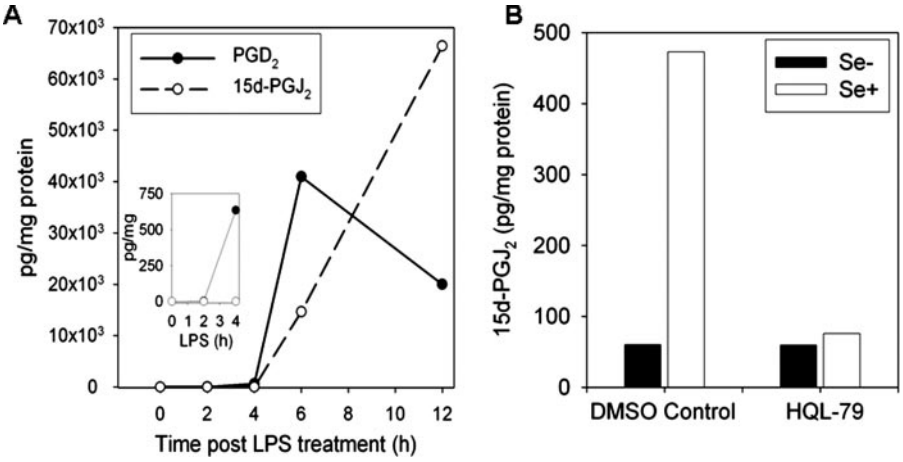


FIGURE 5. **Modulation of $PGD_2$ and 15d-$PGJ_2$ levels in selenium-supplemented macrophages.** *A*, comparison of the production of $PGD_2$ and 15d-$PGJ_2$ in selenium-supplemented BMDM. Culture media supernatants from LPS (1 $\mu$g/ml, 12 h)-treated cells were used to quantitate $PGD_2$ and 15d-$PGJ_2$ using enzyme-linked immunosorbent assay. Results were normalized to the amount of total cellular protein. *Inset*: production of $PGD_2$ at early time points (0–4 h). *B*, effect of HQL-79 on the production of 15d-$PGJ_2$ in RAW264.7 macrophages. Cells were treated with 50 $\mu$M HQL-79 for 12 h followed by LPS stimulation for an additional 12 h. Results shown are representative of three independent experiments.

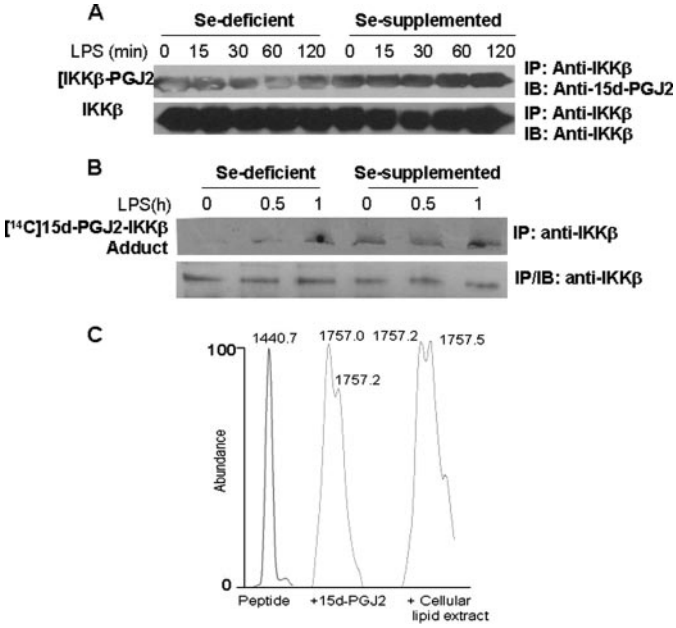


FIGURE 6. **Modification of IKK$\beta$ subunit in selenium-supplemented RAW264.7 cells by 15d-$PGJ_2$.** *A*, lysates from selenium-deficient and selenium-supplemented RAW264.7 cells, stimulated with LPS for 0–2 h, were used to analyze the modification of IKK$\beta$ by 15d-$PGJ_2$ in immunoprecipitation experiments followed by Western immunoblotting. The polyvinylidene difluoride membrane was stripped and reprobed with anti-IKK$\beta$ to normalize for protein loading ($n = 2$). *B*, the selenium-deficient and selenium-supplemented RAW264.7 cells were pretreated with [$^{14}$C]arachidonic acid (1 $\mu$Ci and 30 $\mu$M) for 2 h prior to stimulation with LPS for 1 h. The cell lysates were used to isolate IKK$\beta$ by immunoprecipitation and analyzed on an SDS-PAGE followed by autoradiography ($n = 2$). *C*, mass spectrometric analysis of the interaction of $Me_2SO$, authentic 15d-$PGJ_2$, and total lipid extract from LPS-treated selenium-supplemented macrophages with the IKK$\beta$ peptide.

316.5 $m/z$ units, upon incubation with the cellular lipid extracts similar to that observed with the authentic 15d-$PGJ_2$ standard (Fig. 6*C*). Based on these results, it is clear that endogenous 15d-$PGJ_2$ produced in selenium-supplemented cells modulated the activity of IKK$\beta$ by covalent modification of the essential Cys-179 residue.

*Contribution of COX-1 and COX-2 to the 15d-$PGJ_2$-dependent Regulation of IKK Activity in Selenium-supplemented and Selenium-deficient Macrophages*—The facts that COX-2 is overexpressed in selenium deficiency, but 15d-$PGJ_2$ levels are not increased and IKK$\beta$ activity is not affected are compelling evidences that COX-1 activity may contribute to the formation of 15d-$PGJ_2$. To address this, we utilized selective inhibitors of each of these enzymes. The use of CAY10404, a COX-2-selective inhibitor, at 10-fold higher concentration than the reported $IC_{50}$ values, had no effect on the production of 15d-$PGJ_2$ in LPS-treated selenium-supplemented cells (Fig. 7*A*). In contrast, the use of indomethacin, as a COX-1 inhibitor at 1 $\mu$M, lower than the $IC_{50}$ for COX-2, completely inhibited the production of 15d-$PGJ_2$ in selenium-supplemented cells upon treatment with LPS for 12 h. Identical results were also obtained with another COX-1-selective inhibitor, SC-560 (data not shown). These results suggested that COX-1 plays an important role in the production of the anti-inflammatory 15d-$PGJ_2$. As confirmation, we used the siRNA technology to effectively knockdown the expression of COX-1 in selenium-deficient and selenium-supplemented BMDM cells (Fig. 7*B*). The knockdown of COX-1 was confirmed by Western immunoblotting using COX-1-specific polyclonal antibodies (Fig. 7*B*, *see inset*). Lipopolysaccharide stimulation of COX-1 siRNA-transfected selenium-supplemented cells completely inhibited the production of 15d-$PGJ_2$, similar to that observed with the selenium-deficient cells. Studies performed with RAW264.7 cells also exhibited similar results as that with the BMDM cells (data not shown). Furthermore, the effect of SC-560 on the activity of IKK$\beta$ in selenium-supplemented or selenium-deficient RAW264.7 cells was tested by *in vitro* kinase assays following the pulldown of IKK$\beta$. As shown in Fig. 7*C*, the results clearly indicate that IKK$\beta$ activity was increased in LPS-stimulated selenium-supplemented cells treated with SC-560. In addition, gel mobility shift assays performed in selenium-supplemented and selenium-deficient cells indicated that NF-$\kappa$B activity in SC-560-treated selenium-supplemented cells was increased when compared with the untreated (or vehicle-treated) selenium-supplemented cells. (Fig. 7*D*). These results unequivocally confirm that, in selenium-supplemented cells, where the expression of COX-2 is significantly low, COX-1 plays an important role in the control of gene expression via the generation of 15d-$PGJ_2$, an endogenous activator of anti-inflammatory pathways, targeting such transcription factors as NF-$\kappa$B and PPAR$\gamma$.

*Anti-inflammatory Effects of 15d-$PGJ_2$ Mediated by PPAR$\gamma$-dependent Mechanisms*—We tested the activation of PPAR$\gamma$ as a possible anti-inflammatory pathway in the context of cellular selenium status. Gel mobility shift assays with a consensus dou-

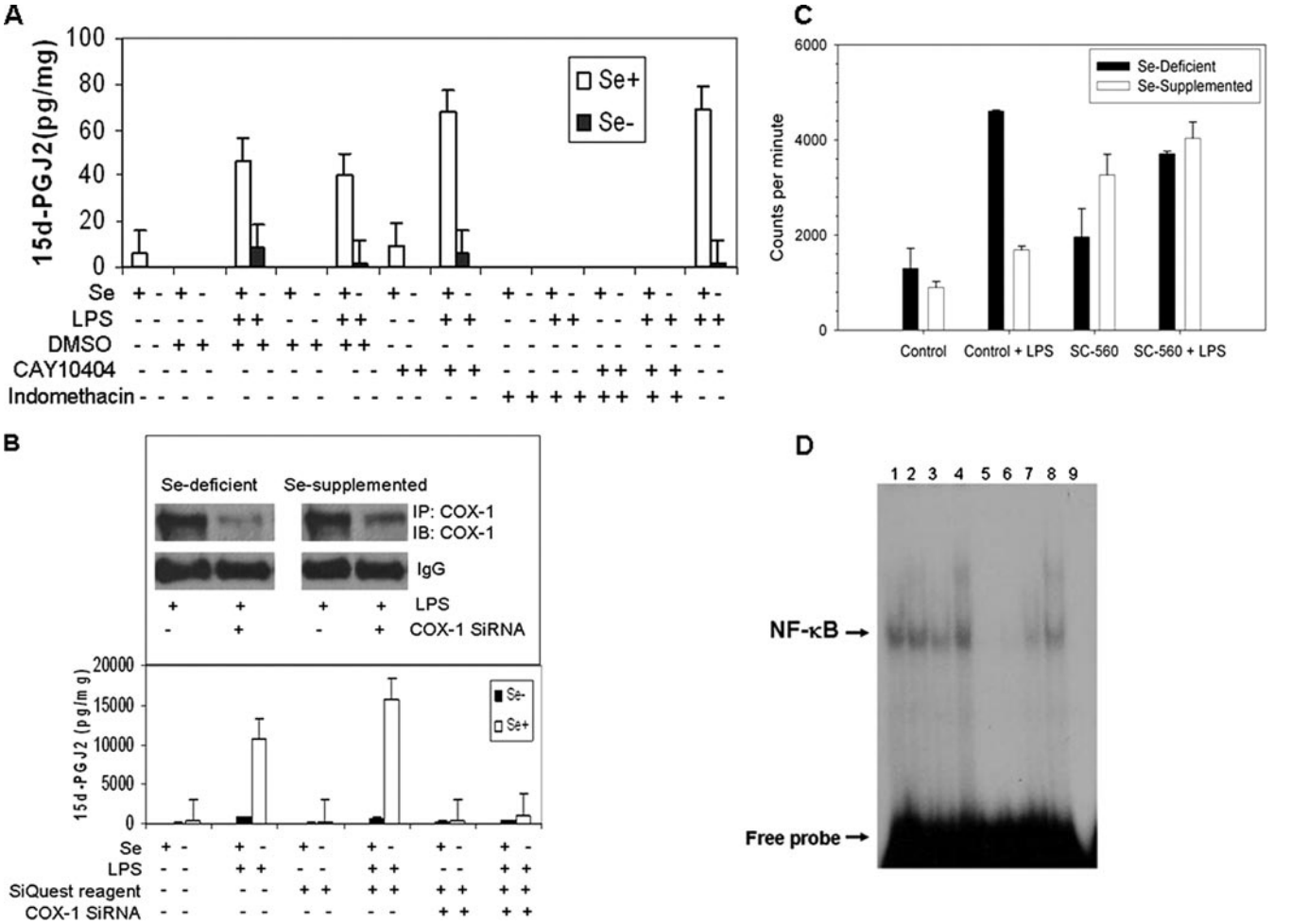


FIGURE 7. **Inhibition of COX-1 in selenium-supplemented macrophages reduces 15d-PGJ$_2$ levels.** *A*, selenium-deficient and selenium-supplemented RAW 264.7 macrophages were pretreated with $Me_2SO$ or indomethacin (1 $\mu$M) or CAY10404 (10 nM) for 24 h followed by stimulation with LPS for an additional 12 h. *B*, selenium-deficient and selenium-supplemented BMDM were transfected with either COX-1 siRNA duplexes or the siQuest reagent alone following which LPS stimulation for 12 h was carried out. The cell lysates were used to determine the levels of COX-1 (*see inset*), whereas the cell culture media supernatants were used to quantify 15d-PGJ$_2$. The values were normalized for total cellular protein. *C*, selenium-supplemented or selenium-deficient RAW264.7 cells were pretreated with $Me_2SO$ or SC-560 for 24 h following which they were stimulated for 2 h with LPS. The IKK$\beta$ isolated from the cell lysates were used in *in vitro* kinase assays with [$\gamma$-$^{32}$P]ATP and GST-I$\kappa$B$\alpha$. *D*, gel mobility shift analysis demonstrating the increased NF-$\kappa$B translocation and binding in selenium deficiency and SC-560-treated selenium-supplemented RAW264.7 cells. *Lanes 1–4* and *5–8* represent $Me_2SO$-treated, $Me_2SO$ plus LPS (2 h), SC-560-treated, SC-560-treated plus LPS (2 h), in selenium-deficient and selenium-supplemented cells, respectively. *Lane 9* represents the cold oligonucleotide competitor. Results shown are representative of $n = 3$ experiments.

ble-stranded PPAR$\gamma$ oligonucleotide showed that the selenium-supplemented cells demonstrated increased binding activity upon stimulation with LPS, whereas the binding in selenium-deficient cells was significantly lower (Fig. 8*A*). Incubation of selenium-supplemented cells with SC-560 followed by stimulation with LPS significantly decreased the translocation and binding activity of PPAR$\gamma$. This was further confirmed in experiments with GW9662, a PPAR$\gamma$ antagonist at 1 $\mu$M, which clearly demonstrated that the repression of COX-2 expression was also mediated by ligand-dependent activation of PPAR$\gamma$ in selenium-supplemented macrophages (Fig. 8*B*). Taken together, these results indicate that the anti-inflammatory effect of selenium is mediated, in part, by 15d-PGJ$_2$ via IKK$\beta$- and PPAR$\gamma$-dependent mechanisms.

## DISCUSSION

Epidemiological studies indicate that deficiency in selenium, although a micronutrient, is common in cigarette smokers (33), individuals with breast cancer (34), HIV patients (35), and those living in geographic locations where the soil selenium levels are low (36). Cardiomyopathy and rhabdomylosis are characteristics of selenium deficiency (37). Recently, supplementation with selenium has been positively correlated with lowered incidences in prostate cancer and HIV replication, where macrophage activation is a crucial step in the inflammatory processes that form the underlying basis of disease progression (38). Here we show that selenium supplementation of macrophages down-regulates the expression of COX-2, via the inhibition of the enzymatic activity of IKK$\beta$ by covalent modification of an essential Cys residue within its activation loop by 15d-PGJ$_2$. To the best of our knowledge, this is the first ever report that links cellular selenium status to the attenuation of NF-$\kappa$B-dependent pro-inflammatory gene expression via the synthesis of 15d-PGJ$_2$.

Consistent with the differential expression of COX isoforms, two patterns, immediate and delayed PG synthesis have been noted (39, 40). Interestingly, the contribution of COX-1 to

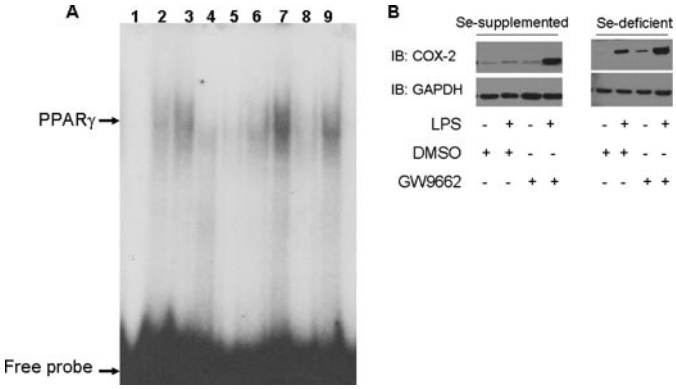


FIGURE 8. **Selenium supplementation activates PPARγ via the production of 15d-PGJ$_2$.** *A*, gel mobility shift analysis demonstrating the PPARγ translocation and binding in selenium-supplemented cells and SC-560-treated selenium-supplemented RAW264.7 cells. *Lanes 2–5* and *6–9* represent Me$_2$SO-treated, Me$_2$SO plus LPS (2 h), SC-560-treated, SC-560 plus LPS (2 h), in selenium-deficient and selenium-supplemented cells, respectively. *Lane 1* represents the cold oligonucleotide competitor. *B*, selenium-supplemented and selenium-deficient RAW264.7 cells were pretreated with GW9662 (1 μM) followed by stimulation with LPS for 2 h. The cell lysates were analyzed for the expression of COX-2 and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) by Western immunoblot analysis. Results shown are representative of $n = 2$ experiments.

delayed PG synthesis has been recently shown to impact the autocrine regulation of tumor necrosis factor-α secretion (41). It is intriguing to note that selenium-deficient cells express COX-2 even prior to LPS stimulation, whereas its expression is attenuated in selenium-supplemented cells. Under such circumstances, the role of COX-1 in arachidonic acid metabolism becomes even more important. Using indomethacin, SC-560, and COX-1 siRNA, we have conclusively shown that COX-1 plays an important role in the activation of NF-κB and consequent expression of pro-inflammatory genes. Our results indicate that arachidonic acid is preferentially utilized by COX-1, even in the presence of reduced levels of COX-2 in selenium-supplemented cells, to eventually produce the anti-inflammatory 15d-PGJ$_2$. However, compared with selenium-supplemented RAW264.7 cells, BMDMs produced 7- to 10-fold higher 15d-PGJ$_2$ upon stimulation with LPS, which is likely due to higher levels of COX-1 expression in the latter cell type (data not shown). *In vivo* studies complement the *in vitro* experiments with regard to increased production of 15d-PGJ$_2$ in the serum of selenium-supplemented mice.

The biogenesis of 15d-PGJ$_2$ has been examined to a much greater extent than any other PG due to its ability to form Michael addition products (24, 42). A preferential time-dependent synthesis of 15d-PGJ$_2$ is seen only in the selenium-supplemented cells. Rossi *et al.* (24) have shown that such an interaction of 15d-PGJ$_2$ with Cys-179 of IKKβ leads to the inhibition of enzymatic activity, without affecting IKKα. Mass spectrometric analysis of the IKKβ peptide, supported by immunoprecipitation and [$^{14}$C]arachidonic acid labeling analysis, clearly validates the previous results of Rossi *et al.* (24) and, in addition, demonstrates the increased interaction of endogenous 15d-PGJ$_2$ with IKKβ in macrophages supplemented with selenium as one of the mechanisms of regulation of NF-κB-dependent gene expression. An immediate effect of such an interaction is the inhibition of IKKβ, in selenium-supplemented cells, resulting in the reduction in the levels of pIκBα. In selenium-deficient cells, higher levels of pIκBα could be due to a feedback activation mechanism of transcription of IκBα by NF-κB followed by phosphorylation. The net effect of such a well regulated process is the enhanced activation of NF-κB in selenium-deficient cells. Apart from interacting with IKKβ, 15d-PGJ$_2$ is an endogenous ligand for PPARγ and may perform the role of an insulin sensitizer without any apparent hepatic, cardiovascular, and hematological toxicities that is commonly seen in the case of the synthetic ligand, rosiglitazone (24). Based on our results, it appears that 15d-PGJ$_2$ is only produced in cells that are sufficient in selenium and have the ability to convert PGH$_2$ to PGD$_2$, which then undergoes two dehydration reactions to be converted to 15d-PGJ$_2$. Although the concentration of selenium used in this investigation is higher than that found in the plasma, results with lower selenium concentrations have also yielded identical results (data not shown). Based on our results, we believe that 15d-PGJ$_2$ is converted via its precursor, PGD$_2$, which suggests a selenium-dependent regulation of the H-PGDS as an important determinant in this process.

In macrophages, activation of PPARγ can repress the transcription of PG isomerases, such as thromboxane synthase via its interaction with the transcription factor, Nrf2 (43). It is possible that a host of transcriptional repression and activation mechanisms could be initiated by 15d-PGJ$_2$. Along these lines, preliminary observations suggest a selenium-dependent down-regulation of thromboxane synthase expression, whereas H-PGDS expression is increased in selenium-supplemented cells (data not shown). It has been shown that COX-2 can functionally couple with microsomal PGE$_2$ synthase-1 to regulate the production of PGE$_2$ (44). Similarly, gain-of-function studies in HEK293 cells have shown that COX-1 and -2 can couple with H-PGDS (45). Studies with HQL-79 clearly indicate that H-PGDS is involved in the increased production of 15d-PGJ$_2$. Indeed, preliminary data suggest that the cellular selenium status can modulate the transcription of PG synthases, which could play a pivotal role in shunting PGH$_2$ either toward pro-inflammation or anti-inflammation (data not shown). Thus, preferential and functional interaction of COX-1 with H-PGDS in selenium-supplemented macrophages, as opposed to COX-2 and microsomal PGE$_2$ synthase-1 in selenium deficiency, could also determine the fate of arachidonic acid toward resolution as a function of cellular selenium status. This observation could have great biological significance given that the balance between the interaction of COX-2 with H-PGDS and PGE$_2$ synthase-1 is a major determinant of atherosclerotic plaque instability in humans (46). Taken together, it is plausible that, in selenium-supplemented cells, COX-1 mainly serves as an enzyme for the synthesis of 15d-PGJ$_2$, which is further aided by the transcriptional regulation of downstream PG synthases and their interaction with specific COX isozymes.

Results of the *in vitro* kinase assays indicate that increased levels of 15d-PGJ$_2$ inhibits the activity of IKK in selenium-supplemented macrophages leading to the decreased activation of NF-κB, which could be reversed by inhibition of COX-1. In contrast, treatment of selenium-deficient cells with COX-1 inhibitor neither affected the activity of IKK or NF-κB activation nor had any effect on the basal levels of 15d-PGJ$_2$ (Fig. 7).

On the other hand, the nuclear translocation of PPARγ clearly showed reduced levels of binding in the absence of COX-1 activity (Fig. 8). Inhibition of PPARγ using GW9662 increased the expression of COX-2 even in the selenium-supplemented cells suggesting that 15d-$PGJ_2$-dependent activation of PPARγ also plays an important role in the modulation of NF-κB-dependent transcription of genes.

In conclusion, we have reported the molecular basis of the anti-inflammatory property of selenium to be driven by modulation of 15d-$PGJ_2$ metabolism. This is the first ever report on the preferential increase of an endogenous anti-inflammatory product of arachidonic acid metabolism as a function of cellular selenium status. As a consequence of increased 15d-$PGJ_2$, the cellular expression of COX-2, in response to inflammatory stimulus, was significantly decreased. Given that 15d-$PGJ_2$ can modify many proteins, a broader question as to how such a selenium-dependent "switch" in arachidonic acid metabolism may play a role in host immunity and inflammation is currently being examined.

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