

Uteroglobin Represses Allergen-induced Inflammatory Response by Blocking PGD₂ Receptor-mediated Functions

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Abstract

Uteroglobin (UG) is an antiinflammatory protein secreted by the epithelial lining of all organs communicating with the external environment. We reported previously that UG-knockout mice manifest exaggerated inflammatory response to allergen, characterized by increased eotaxin and Th2 cytokine gene expression, and eosinophil infiltration in the lungs. In this study, we uncovered that the airway epithelia of these mice also express high levels of cyclooxygenase (COX)-2, a key enzyme for the production of proinflammatory lipid mediators, and the bronchoalveolar lavage fluid (BALF) contain elevated levels of prostaglandin D₂. These effects are abrogated by recombinant UG treatment. Although it has been reported that prostaglandin D₂ mediates allergic inflammation via its receptor, DP, neither the molecular mechanism(s) of DP signaling nor the mechanism by which UG suppresses DP-mediated inflammatory response are clearly understood. Here we report that DP signaling is mediated via p38 mitogen-activated protein kinase, p44/42 mitogen-activated protein kinase, and protein kinase C pathways in a cell type-specific manner leading to nuclear factor- κ B activation stimulating COX-2 gene expression. Further, we found that recombinant UG blocks DP-mediated nuclear factor- κ B activation and suppresses COX-2 gene expression. We propose that UG is an essential component of a novel innate homeostatic mechanism in the mammalian airways to repress allergen-induced inflammatory responses.

Key words: allergic airway inflammation • uteroglobin • Th2 cytokines • eosinophils • secretoglobin

Introduction

Allergic inflammatory disorders result from a complex interplay of genetic and environmental factors. Even though the incidence of these disorders is on the rise throughout the industrialized world, the majority of the population manages to avert allergic inflammatory diseases. Thus, it appears that homeostatic mechanisms may exist to repress allergen-mediated inflammatory responses.

Uteroglobin (UG) is a steroid-inducible, low molecular weight, multifunctional, secreted protein with antiinflam-

matory/antichemotactic properties (for review see reference 1). It is the founding member of a newly designated *Secretoglobin* superfamily of proteins (2), constitutively expressed by the epithelial lining of all organs that communicate with the external environment. Structurally, UG is a homodimer in which the 70-amino acid subunits are covalently linked in antiparallel orientation by two inter-chain disulfide bridges forming a central hydrophobic cavity (3, 4). It has been suggested that this central cavity is capable of binding hydrophobic ligands such as steroid hormones, polychlorinated biphenyls, and retinol. The physiological importance of this property of UG remains unclear.

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Abbreviations used in this paper: AA, arachidonic acid; BALF, bronchoalveolar lavage fluid; COX, cyclooxygenase; EMSA, electrophoretic mobility shift analysis; MAPK, mitogen-activated protein kinase; MG, myoglobin; OVA, ovalbumin; PGD₂, prostaglandin D₂; PKC, protein kinase C; rUG, recombinase UG; UG, uteroglobin.

We reported previously that OVA immunization and challenge of UG-KO mice (5) cause highly exaggerated allergic response characterized by increased expression of Th2 cytokines, eotaxin, and eosinophil infiltration in the lungs (6). This suggests that UG may have a protective role against allergen-induced inflammatory response. However, the molecular mechanism(s) by which UG prevents allergen-induced inflammatory response remains unclear.

It is generally accepted that mast cells and other cell types in the lungs orchestrate allergic inflammation through the production of various cytokines and lipid mediators of inflammation such as prostaglandin D₂ (PGD₂) (7–11). Previous studies have shown that antigen/allergen challenge stimulates the release of arachidonic acid (AA) from membrane phospholipids by cytosolic phospholipase A₂ catalysis (12). Moreover, it has been reported that PGD₂ reinforces low dose antigen-stimulated Th2-type inflammatory responses via expression of macrophage-derived chemokines (13). More recently, it has been demonstrated that targeted overexpression of PGD₂ synthase gene in the lungs of transgenic mice causes elevated levels of Th2 cytokines and eosinophil infiltration (14). Prostaglandins are produced from oxidation of arachidonic acid by cyclooxygenases (COX), also known as prostaglandin H synthase (15). Between the two isoforms of COX, COX-1 is constitutively expressed, whereas COX-2 is agonist inducible and plays critical roles in the production of proinflammatory lipid mediators (for review see reference 16). Moreover, it has been reported that: (a) the expression of COX-2, a critical enzyme for the production of proinflammatory lipid mediators in the respiratory system, is elevated in patients with allergic inflammation and asthma (17); (b) bronchoalveolar lavage fluids (BALFs), after acute antigen challenge (7) and those from patients with allergic asthma, contain high levels of PGD₂; (c) mice in which PGD₂ receptor, DP, is disrupted by gene targeting are refractory to developing allergen-induced airway inflammation (18); and (d) treatment of mice with a synthetic DP antagonist prevents allergic inflammation (19). Together, these results suggest that allergen-induced activation stimulates the release of PGD₂, which mediates inflammation via DP signaling. However, the molecular mechanism(s) of DP signaling leading to allergic inflammatory response and the mechanism(s) by which UG represses allergen-induced inflammatory responses, until now, remained obscure.

To understand the molecular mechanism(s) of the exaggerated allergic response in UG-KO mice, we first performed detailed analyses of inflammatory response in OVA-sensitized and OVA-challenged UG-KO mice. We uncovered that in addition to the elevated expression of eotaxin, Th2 cytokines, and pulmonary eosinophil infiltration reported previously (6), the UG-KO mice also manifest elevated levels of PGD₂ in the BALF and show increased COX-2 gene expression in the lungs. More significantly, treatment of these mice with purified recombinant UG (rUG) markedly inhibits these responses. Using representative cell lines from the mammalian respiratory

system, we conducted experiments to understand: (a) the molecular mechanism(s) of DP signaling and (b) the mechanism by which UG suppresses DP-mediated inflammatory response. The results of these studies show that DP signaling is mediated via p38 mitogen-activated protein kinase (MAPK), p44/42 MAPK, and protein kinase C (PKC) pathways in a cell type-specific manner leading to the activation of nuclear factor (NF)- κ B. Activation of NF- κ B then stimulates the expression of COX-2, an agonist (allergen)-inducible enzyme that is critical for the production of inflammatory lipid mediators. Most importantly, we found that UG binds PGD₂, blocks DP signaling, inhibits NF- κ B activation, and consequently suppresses COX-2 gene expression. Our results, for the first time, demonstrate that at least one of the mechanisms by which DP signaling mediates allergic inflammatory response is by activating NF- κ B that stimulates COX-2 expression. We propose that UG is a critical component of an innate homeostatic mechanism to prevent inadvertent stimulation of allergen-induced, DP-mediated inflammatory response.

Materials and Methods

Cell Lines and Mice. The human bronchial smooth muscle (BSM-2146) and human alveolar type II (A549) cells were from Clonetics-BioWhittaker, Inc. The NIH-3T3 cells were from American Type Culture Collection. UG-KO mice were generated as described previously (5). Both UG-KO and WT mice were maintained under germ-free conditions, and all experiments were performed according to an institutionally approved animal use protocol.

Production and Purification of rUG. The production and purification of rUG have been described previously (20, 21).

Sensitization and Antigen Challenge of WT and UG-KO Mice. UG-KO and their WT littermates were injected i.p. with 10 μ g of OVA (grade V; Sigma-Aldrich) in 0.2 ml alum on day 0 and 14. The sensitized mice were challenged with OVA on day 21 as described previously (6, 14).

Lung Lavage, Tissue Fixation, Histology, and Immunofluorescence. The mice were killed 24 h after the OVA challenge, and BALF was collected in 1 ml of PBS as reported previously (6, 14). Total leukocyte counts and numbers of each cell type per milliliter of BALF were determined as reported (6, 14). Lung tissues were fixed in 4% paraformaldehyde and embedded in paraffin blocks. Tissue sections were stained with Verfuerrth-Luna by American Histolabs, Inc. The stained slides were examined by using a Carl Zeiss MicroImaging, Inc. Axiophot Microscope, and digital photomicrographs were recorded.

RNA Isolation and Northern Blot Analyses. Total RNAs from whole lungs were isolated using either Trizol (Invitrogen) or RNAzol B (Teltest Inc.) following the suppliers' protocol. Total RNA (25–40 μ g) loaded in each lane were resolved by electrophoresis on 1.5% formaldehyde-agarose gels, and RNAs were transferred to Hybond N⁺ (Amersham Biosciences), cross-linked, and hybridized with [α -³²P]-dCTP-labeled cDNA probes at 68°C. The probes were generated by RT-PCR using gene-specific primers (Table S1, available at <http://www.jem.org/cgi/content/full/jem.20031666/DC1>) and sequence was confirmed by DNA sequencing. RNA loading was standardized by hybridizing the blots with GAPDH or β -actin probes (5).

Cytokine and Eotaxin-mRNA Determination by Real-Time Quantitative RT-PCR Analyses. The total RNA (1 µg) from the lungs was reverse transcribed using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). Real-time quantitative RT-PCR was performed using ABI Prism 7000 Sequence Detection System (Applied Biosystems) with cDNA equivalent to 40 ng of total RNA and SYBR Green PCR Master Mix (Applied Biosystems) following the manufacturer's protocol. The following PCR conditions were used: denaturation at 95°C for 10 min followed by 50 cycles of amplification at 94°C for 15 s and 60°C for 1 min. The primers used for IL-4, IL-5, IL-13, eotaxin, and β-actin are presented in Table S1. The data from each PCR run was analyzed using ABI Prism Software version 1.01 (Applied Biosystems). The final data were normalized to β-actin and presented as fold induction compared with the expression level in WT mice. Quantitation was performed using three independent total RNA samples for each treatment group.

Semiquantitative RT-PCR Analyses for DP- and UG-mRNA Expression. The total RNA (1 µg) was reverse transcribed, and semiquantitative PCR was performed to amplify target fragments of cDNA with 5 µl of reverse transcription product using human and mouse DP- and UG-specific sense and antisense primers (Table S2, available at <http://www.jem.org/cgi/content/full/jem.20031666/DC1>). The primers for GAPDH are as reported previously (6). The following PCR conditions were used: denaturation at 95°C for 1 min followed by 30 cycles of amplification at 95°C for 30 s, 60°C for 50 s, 68°C for 3 min, and a final incubation at 68°C for 10 min.

Cell Culture and Treatment. BSM-2146 cells were grown in their specified SmGM-2 medium (Clonetics). The NIH-3T3 cells were grown in DMEM at 37°C with 5% CO₂ as described above. Cells were grown to 70–80% confluence before treating with various effectors (PGD₂, rUG, kinase inhibitors, etc.), washed once with OptiMem-1 containing 2.5% FBS and then treated with the indicated effectors for the indicated period at 37°C with 5% CO₂. The extracellular signal-regulated kinase pathway-specific inhibitor (PD98059) and the p38 MAPK pathway-specific inhibitor (SB203580) were dissolved in DMSO and then added to the OptiMEM-1 to obtain the indicated final concentration. Protein kinase C inhibitor, bisindolyl maleimide III (BMIII), was dissolved in water and added to the medium to obtain the desired final concentrations. Cells were pretreated for 15 min with these inhibitors or with DMSO alone (control) before addition of PGD₂ (up to 50 µM) or as indicated. BSM-2146 cells were incubated for 3 h, and NIH-3T3 cells were incubated for 2 h at 37°C with 5% CO₂.

³H-PGD₂-binding Assay. Cells were grown to 70–80% confluence in their recommended growth medium in 6-well cell culture plates, washed once with OptiMEM-1 without serum, and then incubated with 10 nM ³H-PGD₂ in the absence and presence of varying concentrations (1 nM–5 µM) of PGD₂ for 30 min at 4°C in 3 ml of OptiMEM without serum. Unbound ³H-PGD₂ was removed by washing the cells three times with PBS. Cells were then lysed with lysis buffer, and 300 µl of the cell lysate was applied on a strip of 3-mm Whatman filter paper in a scintillation vial, dried at room temperature, and counted in a scintillation counter.

Transfection of Cells with DP Antisense Oligonucleotides. A phosphorothioate DP antisense oligo (5'-GTGACGGCATTG-CACGAGAAGG-3') was designed to inhibit DP expression. As a control, the corresponding sense-oligo (5'-CCTTCTCGTG-CAATGCCGTCAC-3') was used. When the cells reached 30–

40% confluence, they were rinsed once with serum-free OptiMEM I medium. The oligonucleotides were then delivered to the cells by Oligofectamine™ Reagent (Invitrogen) according to the manufacturer's instructions. After 28 h, the cells were stimulated with 1 µM PGD₂, and total proteins were isolated. The relative levels of COX-2 protein were estimated by Western blot analysis.

Western Blot Analyses. For detection of the phosphorylated proteins, cell lysates were prepared in presence of protease and protein phosphatase inhibitors. Total protein (40 µg) from each sample was resolved by electrophoresis using 7.5% or 12% SDS-polyacrylamide gels under reducing conditions and electrotransferred to polyvinylidene fluoride membrane (Immobilon P; Millipore Corporation). Immunoblot analysis was performed using either goat or rabbit polyclonal anti-COX-2 (Santa Cruz Biotech Inc.)/anti-phospho p38-MAPK/anti-phospho p44/42 antibodies (Cell Signaling), respectively. Horseradish peroxidase-conjugated anti-goat or anti-rabbit IgGs were used as the second antibody. Chemiluminescent detection was performed by using ECL system (Amersham Biosciences) according to the manufacturer's protocol. Total protein loading standard, β-actin, was detected using Actin (Ab-1) Kit (Oncogene Research Products) according to the protocol of the supplier.

Blocking DP-mediated COX-2 Expression by DP Monoclonal Antibody Treatment of the Cells. The NIH-3T3 cells were grown to 70–80% confluence. The cells were washed three times with serum-free OptiMEM and incubated with DP monoclonal antibody (Abcam, UK) in varying dilutions at room temperature with gentle agitation. After washing the cells with serum-free OptiMEM, the cells were stimulated with 1 µM PGD₂ for 2 h. Total RNA was isolated and analyzed for COX-2 mRNA expression by real-time quantitative RT-PCR (22) using the mouse COX-2-specific primers (Table S2). Fold COX-2 expression levels were normalized to 18S rRNA (for 18S primers see Table S2) and compared with the average values in untreated control cells. All determinations were performed in triplicates, and each experiment was repeated at least twice. COX-2 protein expression was determined by Western blot analysis.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay. Cells were treated with PGD₂ (50 µM) in the presence and absence of rUG (150 nM). Nuclear extracts were prepared using commercially available buffers (GENEKA Biotech Inc.) using supplier's protocol. Electrophoretic mobility shift analyses (EMSA) were performed using the nuclear extracts (20 µg protein) on a non-denaturing 5% polyacrylamide gel with the following oligonucleotides: for human cell extracts, hNFκBwt, 5'-AGTTGAGGGG-ACCTTCCCAGGC-3'; hNFκBmu, 5'-AGTTGAGATGACT-CCGCCAGGC-3'; for mouse cell extracts, mNFκBwt, 5'-GAGGGTGAGGGGATCCCTTAGTTAGGAC-3'; mNFκBmu, 5'-GAGAGGTGAGATGATAGCCTTAGTTAGGAC-3'; mNF-IL6, 5'-CTGCCGCTGCGGTTCTTGCGCAACTCACT-3'; mAPI1, 5'-AAAGACAGAGTCACCACTAATT-3'. NF-κB double-stranded oligonucleotides were generated by annealing sense and antisense oligonucleotides without and with mutations (bold). Specificity of protein-DNA complexes was verified either by competing with cold NF-κBwt/NF-κBmu oligos or by immunoreactivity with polyclonal antibodies specific for p65/p50 subunits of NF-κB.

Statistical Analysis. Data were analyzed by using GraphPad Prism software (version 3.03). Student's *t* test was used to determine the significant differences between the means; *p*-values of <0.05 were considered as statistically significant.

Results

UG Inhibits Th2 Cytokine Expression in Allergen-sensitized and -challenged UG-KO Mice. We reported previously that UG-KO mice, sensitized and challenged with OVA, manifest exaggerated allergic response characterized by elevated eotaxin and Th2 cytokine expression and an increased number of eosinophils in BALF (6). In this study, we examined the effects of rUG on these parameters. We first treated the OVA-sensitized UG-KO mice with rUG before OVA challenge and determined the expression of eotaxin and representative Th2 cytokine mRNAs by real-time quantitative RT-PCR. Our results confirm that OVA sensitization and challenge increase the level of these mRNAs in the lungs of UG-KO mice (Fig. 1 A) as reported previously (6). More importantly, we demonstrate that rUG

treatment of OVA-sensitized UG-KO mice before OVA challenge inhibits the expression of IL-4 (Fig. 1 A, i), IL-5 (Fig. 1 A, ii), IL-13 (Fig. 1 A, iii), and eotaxin (Fig. 1 A, iv), all of which are recognized markers of allergic inflammatory response. The reduced level of inflammatory response in the lungs of OVA-sensitized and challenged WT mice may be due to the stimulation of UG expression in response to increased levels of Th2 cytokines. This hypothesis is at least partially supported by the fact that IL-13 treatment stimulates UG production in rat lungs (23). Therefore, we analyzed UG mRNA and UG protein in the lungs of OVA-sensitized and challenged WT mice by semiquantitative RT-PCR and immunofluorescence, respectively. The results show that OVA sensitization and challenge of WT littermates of UG-KO mice expressed higher levels of

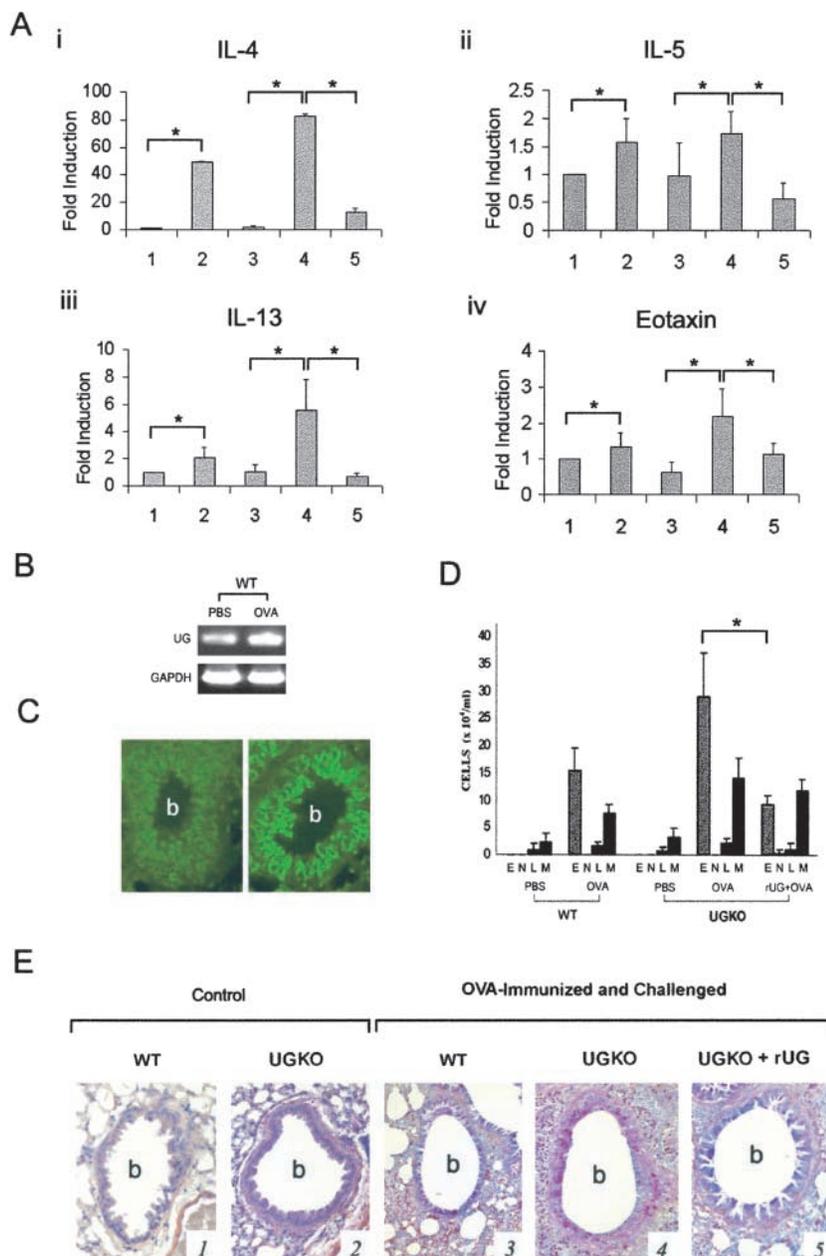


Figure 1. UG Inhibits proinflammatory cytokine expression and eosinophil infiltration. (A) The expression of IL-4 (i), IL-5 (ii), IL-13 (iii), and eotaxin (iv) mRNA was determined by real-time quantitative RT-PCR using total RNA from the lungs of PBS-treated WT mice (bar 1), OVA-sensitized and -challenged WT mice (bar 2), UG-KO mice (bar 3), OVA-sensitized and -challenged UG-KO mice without rUG treatment (bar 4), and OVA-sensitized UG-KO mice that were pretreated with rUG (250 μ g of rUG in 200 μ l PBS administered i.v.) 30 min before OVA challenge (bar 5). Asterisks indicate significance at $P < 0.05$. (B) UG-mRNA expression in the lungs of PBS-challenged WT and OVA-sensitized and -challenged WT mice by semiquantitative RT-PCR. (C) UG protein expression (immunofluorescence) in bronchiolar epithelial cells of WT mouse challenged with PBS (left) or sensitized and challenged with OVA (right). (D) Total cell counts and number of individual leukocytes per milliliter of BALF from PBS (control) and OVA-sensitized and -challenged WT (left) and UG-KO (right) mice and the results from OVA-sensitized and challenged (middle) and OVA-sensitized UG-KO mice treated with rUG before OVA challenge (extreme right). Values are expressed as the mean \pm SD ($n = 5$ per treatment group). E, eosinophil; N, neutrophil; L, lymphocyte; M, monocyte. (E) Eosinophil infiltration in nonsensitized and unchallenged WT control (frame 1); nonsensitized and unchallenged UG-KO control (frame 2); WT mouse sensitized and challenged with OVA (frame 3); UG-KO mouse sensitized and challenged with OVA (frame 4); UG-KO mouse sensitized and treated with rUG before OVA challenge (frame 5). b, bronchiole. Magnification, $\times 400$.

UG mRNA (Fig. 1 B) and UG protein (Fig. 1 C) compared with their nonsensitized and nonchallenged counterparts. These results raise the possibility that the observed OVA-induced elevation of UG production in WT mouse lungs is likely to be a homeostatic response to counteract inadvertent stimulation of allergen-induced inflammation.

UG Inhibits Eosinophil Infiltration. We further determined the effects of rUG treatment on the infiltration of various leukocytes including eosinophils in the BALFs of these mice. Quantitation of each cell type in the BALF show that although leukocytes were virtually undetectable in control WT and UG-KO mice (Fig. 1 D); OVA sensitization and challenge caused an upward shift not only in the total number of infiltrated cells but also in the cell types. Most importantly, rUG treatment of OVA-sensitized UG-KO mice before OVA challenge significantly inhibited the number of eosinophils in BALF (Fig. 1 D). These results demonstrate that UG is a potent inhibitor of allergen-induced lung infiltration of leukocytes, especially eosinophils.

Histological analysis of the lungs of OVA-sensitized and OVA-challenged UG-KO and WT mice for eosinophil infiltration show that eosinophils in the lungs of control WT and UG-KO mice are barely detectable (Fig. 1 E, frames 1 and 2). In contrast, although only a slight increase in the level of eosinophil infiltration was detectable in the lungs of OVA-sensitized and -challenged WT mice (Fig. 1 E, frame 3), it was markedly elevated in those of the UG-KO mice (Fig. 1 E, frame 4). Most importantly, when the OVA-sensitized UG-KO mice were treated with rUG before OVA challenge, it markedly inhibited eosinophil infiltration in the lungs (Fig. 1 E, frame 5). These results demonstrate that UG is a potent inhibitor of allergen-induced eosinophil infiltration in the lungs.

UG Inhibits Allergen-Induced Elevation of PGD₂ Levels in BALF. Elevated levels of PGD₂ in the BALF of patients with allergic asthma have been reported, and it has been suggested that PGD₂ is a potent chemoattractant for eosinophils (11, 24). Thus, we sought to determine the level

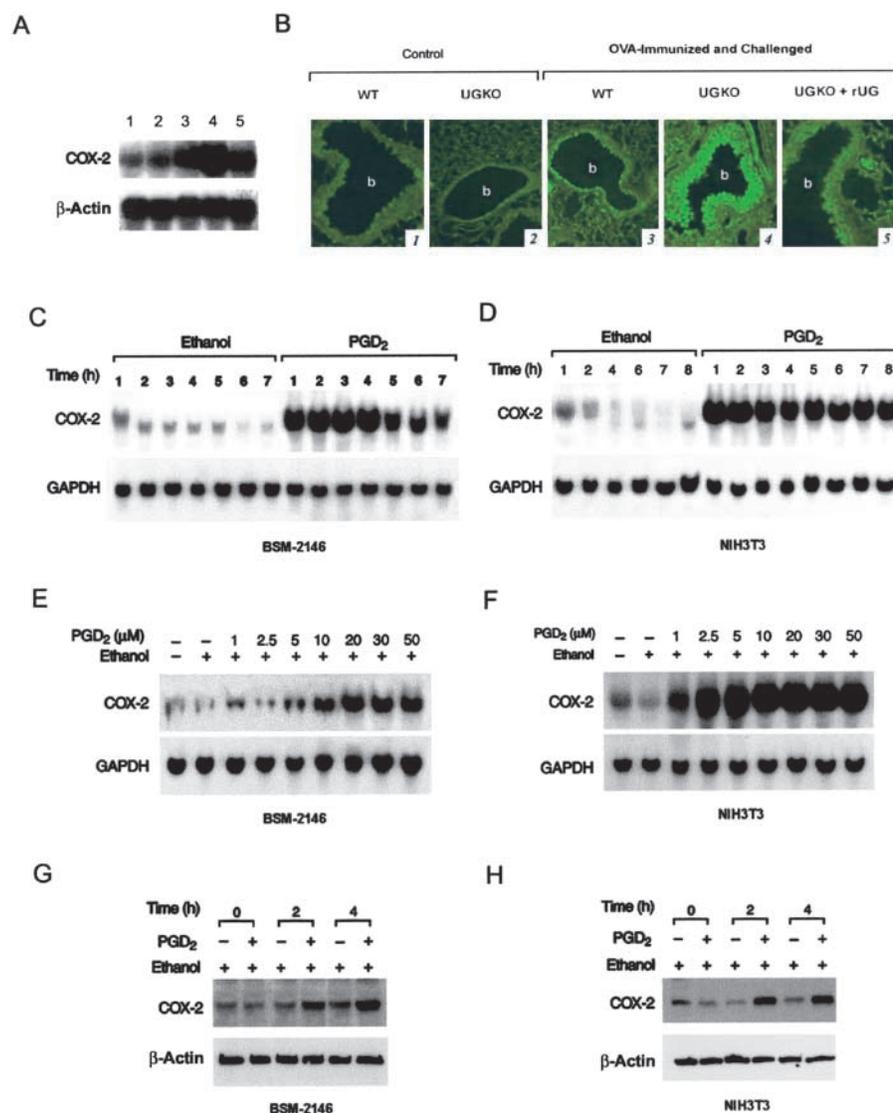


Figure 2. UG inhibits COX-2 expression induced by allergens in vivo and by PGD₂ in vitro. (A) Northern blot analysis of COX-2 mRNA expression in the lungs of control WT (lane 1) and control UG-KO (lane 2), OVA-sensitized and -challenged WT (lane 3), OVA-sensitized and -challenged UG-KO (lane 4), and OVA-sensitized and rUG-treated before OVA challenge of UG-KO (lane 5) mice. (B) COX-2 immunofluorescence in representative control WT (frame 1) and control UG-KO (frame 2) mice; WT mouse sensitized and challenged with OVA (frame 3); UG-KO mouse sensitized and challenged with OVA (frame 4); UG-KO mouse sensitized and treated with rUG before OVA challenge (frame 5). b, bronchiole. Magnification, ×400. Time-course of PGD₂-induced COX-2 mRNA expression in BSM-2146 (C) and NIH-3T3 (D) cells. Dose-response of PGD₂-induced stimulation of COX-2 mRNA expression in BSM-2146 (E) and NIH-3T3 (F) cells. Western blot analysis demonstrating PGD₂-induced COX-2 protein expression in BSM-2146 (G) and NIH-3T3 cells (H).

of PGD₂ in the BALF of OVA-sensitized and -challenged UG-KO and WT mice. In addition, we studied the effects of rUG treatment on PGD₂ levels in BALF of OVA-sensitized and challenged mice. The results (Table S3, available at <http://www.jem.org/cgi/content/full/jem.20031666/DC1>) show that a very low level of PGD₂ is detectable in the BALF of both UG-KO and WT control mice. In contrast, although PGD₂ levels in BALF of OVA-sensitized and challenged WT littermates increased moderately from the baseline, higher levels of PGD₂ were detected in those of the OVA-sensitized and challenged UG-KO mice. Most importantly, PGD₂ levels in the BALF of OVA-sensitized UG-KO mice that were treated with rUG before OVA challenge were significantly reduced compared with those without rUG pretreatment (Table S2). These results suggest that UG inhibits allergen-induced elevation of PGD₂ levels in the lungs.

UG Inhibits Allergen-induced Stimulation of COX-2 Gene Expression In Vivo. COX-2 gene expression in the lungs of allergic asthma patients is markedly elevated (17). Since COX-2 is an agonist-inducible enzyme that catalyzes the production of proinflammatory lipid mediators, we first analyzed COX-2 gene expression in the lungs of nonsensitized and unchallenged UG-KO and WT littermates. The results show that a low level of COX-2 mRNA expression is detectable in the lungs of the WT littermates (Fig. 2 A, lane 1) and in those of the UG-KO (Fig. 2 A, lane 2) mice. In contrast, although COX-2 mRNA expression in the

lungs of OVA-sensitized and challenged WT mice increased slightly (Fig. 2 A, lane 3), the expression levels in UG-KO mice were markedly elevated (Fig. 2 A, lane 4). Most importantly, treatment of OVA-sensitized UG-KO mice with rUG before OVA challenge showed an appreciable inhibition of COX-2 mRNA expression (Fig. 2 A, lane 5). We also found that compared with the intensity of COX-2 immunofluorescence in the bronchioles of control WT and UG-KO mice (Fig. 2 B, frames 1 and 2) immunofluorescence of the OVA-sensitized and challenged WT mice were only slightly elevated (Fig. 2 B, frame 3). However, COX-2 immunofluorescence was strikingly intense in the bronchioles of OVA-sensitized and challenged UG-KO mice (Fig. 2 B, frame 4). Significantly, the intensity of COX-2 immunofluorescence in OVA-sensitized UG-KO mice, treated with rUG before OVA challenge was markedly less intense (Fig. 2 B, frame 5), suggesting that UG suppresses allergen-induced stimulation of COX-2 gene expression in the lungs.

PGD₂ Stimulates COX-2 Gene Expression In Vitro. It has been reported that acute antigen challenge leads to increased PGD₂ levels in human airways (7). Moreover, allergen-induced inflammation in asthmatic airways is associated with high levels COX-2 expression (17). Further, in DP-null mice allergen challenge do not elicit airway inflammation (18). The results of our present study show elevated levels of PGD₂ in the BALF (Table S3) and high level of COX-2 gene expression in the lungs of OVA-sensitized

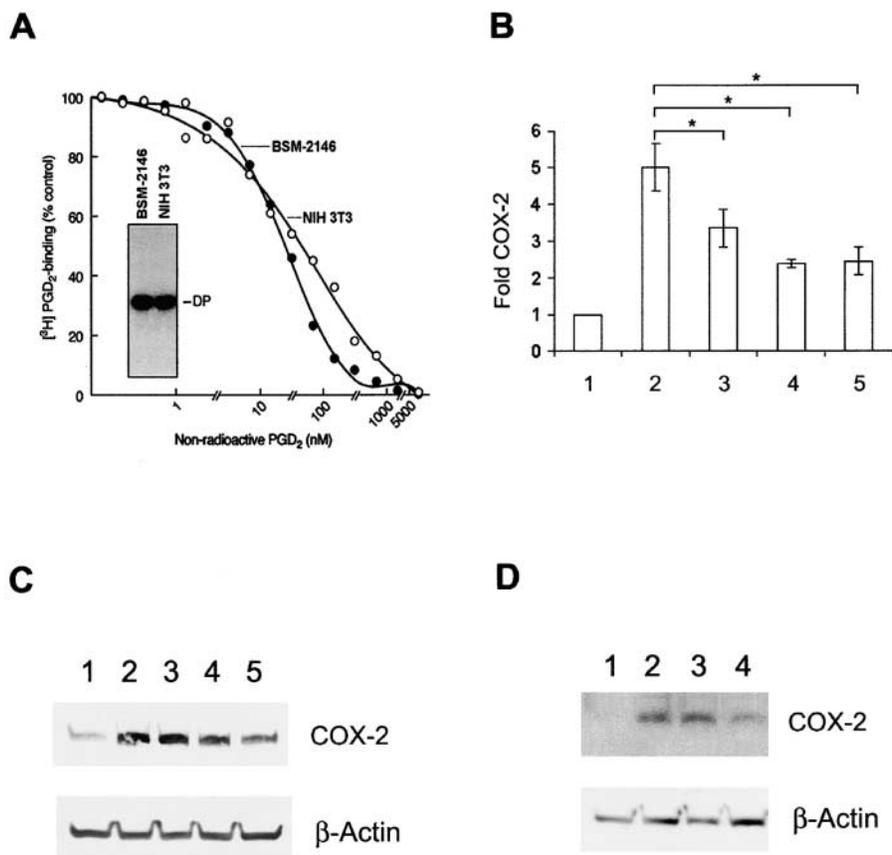


Figure 3. PGD₂-stimulated COX-2 expression is mediated via DP. DP-mRNA expression determined by RT-PCR analysis followed by hybridization with the respective mouse and human DP-cDNA probes (A, inset) in BSM 2146 (lane 1) and NIH-3T3 cells (lane 2). ³H-PGD₂ binding (A) to BSM 2146 (●) and NIH-3T3 (○) cells. Note unlabeled PGD₂ displaces bound ³H-PGD₂ in a dose-dependent manner. Analysis of COX-2 mRNA expression by real-time quantitative RT-PCR (B). NIH-3T3 cells were treated with DP monoclonal antibody before treatment with PGD₂. Results are expressed as the mean of three independent experiments ± SD. Asterisks indicates significance at P < 0.05. Bar 1, untreated control cells; bar 2, PGD₂-treated cells. Prior to PGD₂ stimulation, cells were treated with DP monoclonal antibody at varying dilutions: bar 3, 1:5,000; bar 4, 1:1,000, and bar 5, 1:100. COX-2 protein expression by Western blot analysis (C). Top row: lane 1, untreated control; lane 2, PGD₂-treated cells; lane 3, cells treated with DP monoclonal antibody at 1:5,000 dilution (lane 3); 1:1,000 dilution (lane 4); and 1:100 dilution (lane 5). Inhibition of COX-2 protein expression in cells treated with DP antisense s-oligonucleotide (D). Lane 1, untreated control; lane 2, cells treated with PGD₂; lane 3, cells treated with nonspecific s-oligo; and lane 4, cells treated with DP antisense s-oligo before PGD₂ stimulation.

and challenged UG-KO mice (Fig 2, A and B). Thus, we sought to determine if there is a link between the elevated levels of PGD₂ and COX-2 gene expression. We used three representative cell types (i.e., epithelial, smooth muscle, and fibroblast cells) of the mammalian respiratory system. These cell types were used because these cell types, in cooperation with the immune system, orchestrate the initiation and propagation of allergen-induced inflammatory responses (25–27). We treated the cells with varying concentrations of PGD₂ and determined COX-2 mRNA and protein expression by Northern and Western blot analyses, respectively. The results show that PGD₂ stimulates COX-2 mRNA expression and that this stimulation is time (Fig. 2, C and D) and dose dependent (Fig. 2, E and F). The results of Western blot analyses show that PGD₂ stimulates COX-2 protein expression in both NIH-3T3 and BSM-2146 cells (Fig. 2, G and H). Consistent with these results, we also found that PGD₂ stimulates COX-2 mRNA expression in A549 cells (not depicted). Treatment of the cells with ethanol, used as solvent for PGD₂, did not stimulate COX-2 expression. Together, our results indicate that PGD₂ acts as a potent stimulant for COX-2 expression in three cell types of the respiratory system.

PGD₂-Stimulated COX-2 Expression is Mediated via DP.
It has been demonstrated that PGD₂ mediates allergen-induced airway inflammation via its receptor, DP (18). In this study, we found that PGD₂ stimulates COX-2 expression in three different cell types of the respiratory system. To understand the mechanism(s) of PGD₂-stimulated COX-2 gene expression and inflammatory lipid mediator production, we first determined DP expression and PGD₂ binding using BSM-2146 and NIH-3T3 cells. The results of semi-quantitative RT-PCR analyses show that these cells express DP-mRNA (Fig. 3 A, inset). We also performed competition-binding assays using ³H-PGD₂ as the ligand. The results show that ³H-PGD₂ binding on these cells is saturable, and it is displaced by nonradioactive PGD₂ in a dose-dependent manner (Fig. 3 A). Since it has been reported that a chemoattractant receptor, CRTH2, also binds PGD₂ in some cell types and may transduce some of the biological effects of this eicosanoid, we tested the expression of CRTH2-mRNA in these cells. The results show that the CRTH2-mRNA level is virtually undetectable (not depicted), indicating that CRTH2 is unlikely to be the predominant pathway for PGD₂ signaling in these cell types.

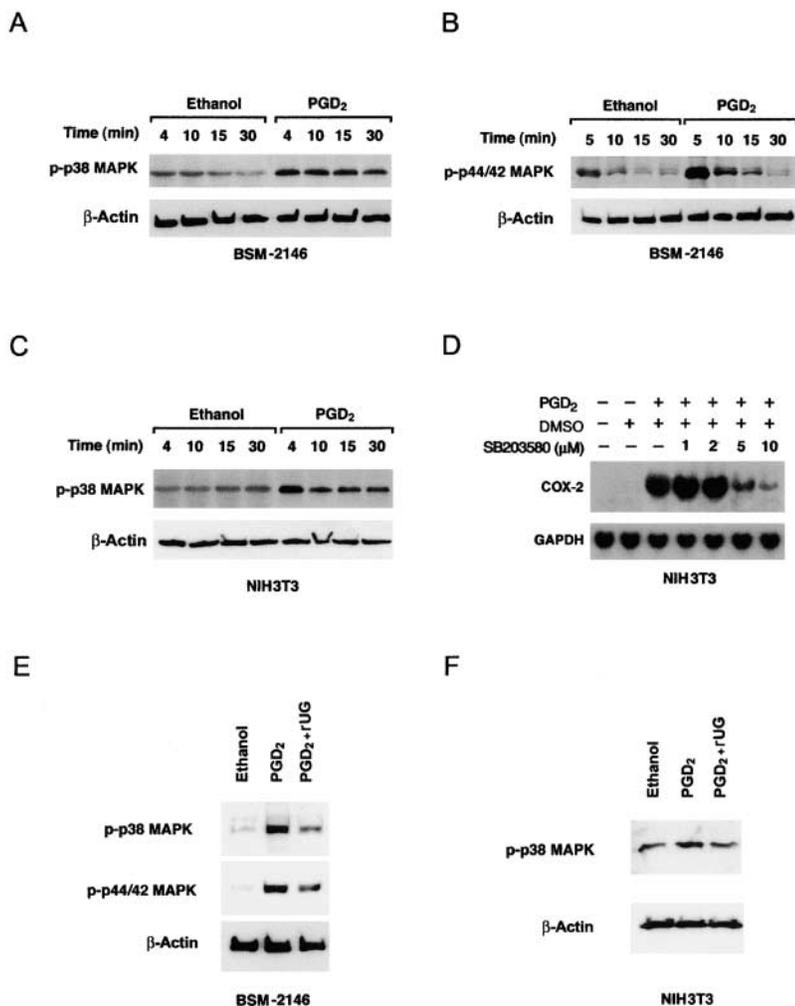


Figure 4. Molecular mechanism(s) of DP signaling is cell type specific. PGD₂ stimulation of BSM-2146 cells induces tyrosine phosphorylation of p38 (A) and p44/42 (B), whereas in NIH-3T3 cells such treatment leads to tyrosine phosphorylation of only p38 MAPK (C). Moreover, p38 MAPK inhibitor, SB203580, inhibits PGD₂-induced COX-2 mRNA expression in a dose-dependent manner (D). In BSM-2146 cells, rUG (1 μM) treatment inhibits PGD₂-mediated phosphorylation of both p44/42 and p38 MAPK (E), and in NIH-3T3 cells rUG inhibits phosphorylation of only p38 MAPK (F).

To confirm whether PGD₂-stimulated COX-2 expression is mediated via DP, we treated the NIH-3T3 cells with DP monoclonal antibody and stimulated these cells with PGD₂. We analyzed both COX-2 mRNA and COX-2 protein expression by real-time quantitative RT-PCR and Western blot analyses, respectively. The results show that although a low level of COX-2 mRNA expression is detectable in PGD₂-untreated control cells (Fig. 3 B, bar 1), PGD₂ treatment stimulates markedly higher levels of COX-2 expression (Fig. 3 B, bar 2). In contrast, COX-2 expression in cells treated with three different dilutions of DP monoclonal antibody before PGD₂ stimulation was significantly reduced (Fig. 3 B, bars 3–5). Consistent with these findings, the results of Western blot analysis show that the PGD₂ untreated cells produced virtually no COX-2 protein (Fig. 3 C, top row, lane 1); however, those treated with PGD₂ markedly stimulated COX-2 protein expression (Fig. 3 C, top row, lane 2). In contrast, cells treated with three different dilutions of DP monoclonal antibody showed a marked reduction in COX-2 protein in a dose-dependent manner (Fig. 3 C, top row, lanes 3–5). To further confirm that PGD₂-stimulated COX-2 expression is DP mediated, we treated the cells with DP antisense s-oligonucleotide before PGD₂ stimulation. The results show that although COX-2 protein expression in cells without PGD₂ treatment is undetectable (Fig. 3 D, lane 1), in PGD₂-treated cells (Fig. 3 D, lane 2) and in cells treated with sense s-oligo before PGD₂ stimulation (Fig. 3 D, lane 3) it is readily detectable. Moreover, marked inhibition of COX-2 protein expression was observed in cells that are first treated with DP antisense s-oligo before PGD₂ treatment (Fig. 3 D, lane 4). Together, these results provide strong evidence that the stimulation of COX-2 expression by PGD₂ is mediated via DP.

Molecular Mechanism(s) of DP-Signaling is Cell Type Specific. Although our results demonstrate that PGD₂ stimulates COX-2 expression via DP, the molecular mechanisms of DP signaling, until now, remained unclear. Thus, we studied the mechanism(s) of DP signaling using our in vitro experimental system. The prostanoids signal through specific G protein-coupled receptors, and it has been reported that many agonists of these receptors activate the MAPK pathway (28). Thus, we analyzed the phosphorylation-dependent activation of kinase pathways. We treated the cells with PGD₂ for varying lengths of time and analyzed tyrosine phosphorylation of MAPK by Western blot analysis using anti-phospho p38-MAPK (Tyr-182) and anti-phospho p44/42-MAPK (Tyr-204) antibodies. PGD₂ stimulates the phosphorylation of both p38- (Fig. 4 A) and p44/42-MAPK (Fig. 4 B) in BSM-2146 cells, whereas in NIH-3T3 cells PGD₂ stimulates the phosphorylation of p38 (Fig. 4 C) but not p44/42 MAPK (not depicted). These results indicate that DP signaling is mediated via the p38 and p44/42 MAPK pathways in a cell type-specific manner. We further confirmed the above findings by analyzing the effects of specific p38 MAPK inhibitor, SB203580, at varying concentrations, on PGD₂-induced COX-2 expression in NIH-3T3 cells. The results show that SB203580 inhibits PGD₂-

induced COX-2 mRNA expression in a dose-dependent manner (Fig. 4 D). Most importantly, we found that rUG treatment inhibits PGD₂-stimulated tyrosine phosphorylation of both p38 and p44/42 MAPK in BSM-2146 (Fig. 4 E) and p38 MAPK in NIH-3T3 cells (Fig. 4 F).

DP Signaling Is Mediated via p38, p44/42, and PKC-activating NF-κB and COX-2 Expression. To determine the possible involvement of other kinase pathways in DP signaling, we treated these cells with PGD₂ in the absence and presence of highly selective inhibitors of PKC (BMIII), p38 (SB203580), and p44/42 MAPK (PD98059), and determined COX-2 mRNA expression by Northern blot analysis. We found that in BSM-2146 cells the inhibitors of all three kinases suppressed PGD₂-mediated stimulation of COX-2 mRNA expression at varying degrees (Fig. 5 A), suggesting that in these cells DP signaling is mediated via activation of p38 and p44/42 MAPK and PKC pathways. In contrast, PGD₂-mediated stimulation of COX-2 expression in NIH-3T3 cells was inhibited only by p38 MAPK inhibitor, SB203580 (Fig. 5 B), whereas in A549 cells only the PKC-specific inhibitor (BMIII) suppressed such stimulation (not depicted).

Current evidence indicates that both MAPK and PKC are capable of activating NF-κB, a pivotal transcription factor that regulates many genes that are critical for inflammation and immunity (29). Therefore, we first examined the effects of specific inhibitors of NF-κB, pyrrolidine dithiocarbamate and NF-κB SN50, on PGD₂-stimulated COX-2 mRNA expression. These agents are effective in inhibiting PGD₂-mediated stimulation of COX-2 mRNA expression in both BSM-2146 (Fig. 5 C) and NIH-3T3 (Fig. 5 D) cells in a dose-dependent manner. These results suggest that DP-mediated COX-2 expression requires NF-κB activation. To determine whether the aforementioned kinase inhibitors inhibit DNA binding activity of NF-κB, we also conducted EMSAs to determine the effect of SB203580, PD98059, and BMIII on PGD₂-stimulated NF-κB activation in both NIH-3T3 and BSM-2146 cells. The results show that although in BSM-2146 cells all three inhibitors suppress the binding of NF-κB probe (Fig. 5 E), in NIH3T3 cells only SB203580 was inhibitory (Fig. 5 F). Further, to confirm that NF-κB activation is required for the PGD₂-stimulated COX-2 expression, we performed the COX-2 promoter-reporter (luciferase) activation assays by transfecting NIH-3T3 cells with reporter constructs, pGL2-COX2WT or pGL2-COX2MU (30), containing WT and mutant NF-κB binding site, respectively. Cells transfected with the vector alone served as controls. The results show that although PGD₂ stimulates luciferase activity in the cells transfected with the pGL2-COX2WT construct, cells transfected with pGL-COX2MU construct had markedly reduced level of luciferase activity (Supplemental Materials and Methods and Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20031666/DC1>). Together, these results indicate that DP signaling is mediated via p38 and p44/42 MAPK and PKC pathways in a cell type-specific manner leading to NF-κB activation, required for COX-2 gene expression.

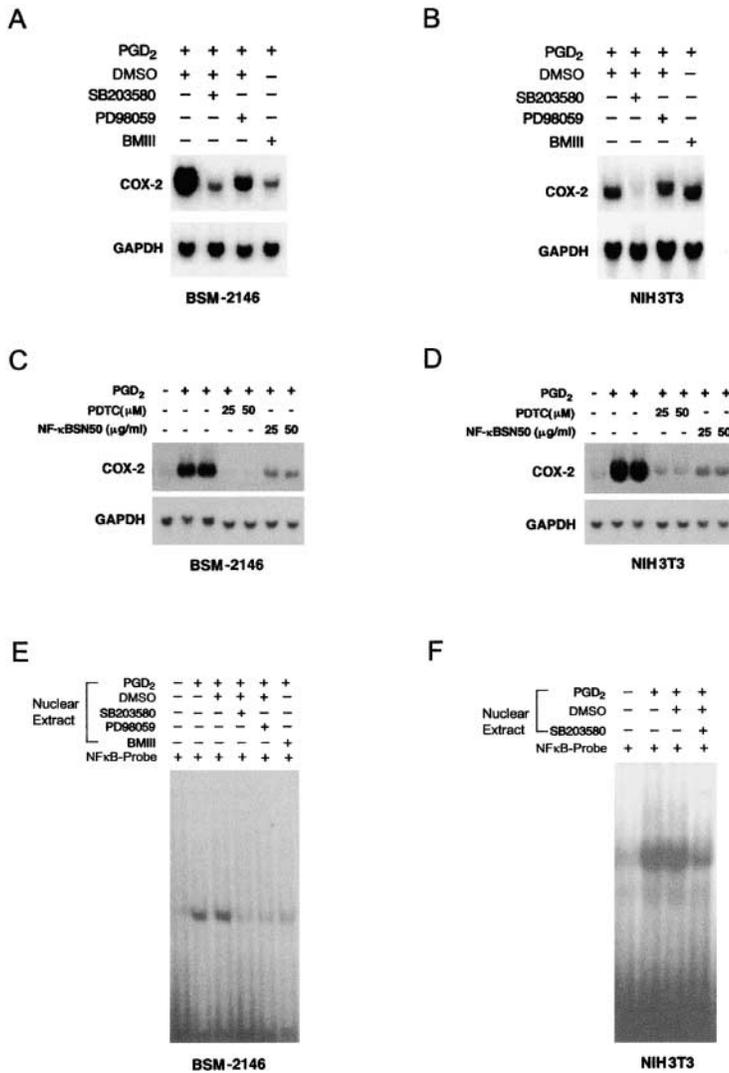


Figure 5. Inhibitors of p38, p44/42, and PKC suppresses DP-mediated NF-κB activation and COX-2 expression. Expression of COX-2-mRNA in BSM-2146 (A) and in NIH-3T3 (B) cells in the absence or presence of inhibitors of p38 MAPK (SB203580, 10 μM), p44/42 MAPK (PD98059, 20 μM), or PKC (BM III, 10 μM). Dose-response of NF-κBNS50 and PDTDC on PGD₂-stimulated COX-2 mRNA expression in BSM-2146 (C) and NIH-3T3 (D) cells. The effect of these inhibitors on NF-κB binding in BSM-2146 (E) and NIH-3T3 (F).

UG Inhibits PGD₂-Stimulated COX-2 Gene Expression In Vitro. We have shown that UG counteracts allergen-induced COX-2 gene expression in OVA-challenged allergic model of UG-KO mice (see the *UG Inhibits Allergen-induced Stimulation...*). Since OVA induces elevated levels of PGD₂ in BALF, we determined if UG inhibits PGD₂-mediated stimulation of COX-2 expression in vitro. We treated BSM-2146 and NIH-3T3 cells with PGD₂ in the presence and absence of varying concentrations of UG and determined COX-2 mRNA expression by Northern blot analysis. We found that in both BSM-2146 (Fig. 6 A) and NIH-3T3 (Fig. 6 B) cells UG inhibits PGD₂-induced COX-2 mRNA expression in a dose-dependent manner. Myoglobin (MG), a nonspecific control does not inhibit PGD₂-stimulated COX-2 expression. Together, these results suggest that UG specifically inhibits PGD₂-mediated COX-2 expression.

UG Inhibits COX-2 Expression by Suppressing PGD₂-stimulated NF-κB Activation. It has been reported that the COX-2 gene expression is regulated by at least three ma-

ajor nuclear factors (i.e., NF-κB, NF-IL6, and AP-1) located in the 5' regulatory region of this gene. Since our results show that PGD₂-stimulated COX-2 expression is mediated via NF-κB activation, we determined whether UG treatment of the cells blocks PGD₂-mediated activation of this transcription factor. We performed EMSAs to identify and confirm the nuclear factor(s) specifically activated by PGD₂ treatment of the cells leading to COX-2 expression. The results show that among the three major nuclear factors PGD₂ specifically activates NF-κB (Fig. 6 C) in a time-dependent manner (Fig. 6 D). To determine whether UG blocks PGD₂-induced NF-κB activation, we performed EMSA using nuclear extracts from NIH-3T3 and BSM-2146 cells treated with PGD₂ in the presence and absence of UG. The results show that UG specifically inhibits DP-mediated activation of NF-κB in both BSM-2146 (Fig. 6 E) and NIH-3T3 (Fig. 6 F) cells. Our results provide strong evidence that UG inhibits PGD₂ receptor-mediated inflammation by blocking the activation of NF-κB.

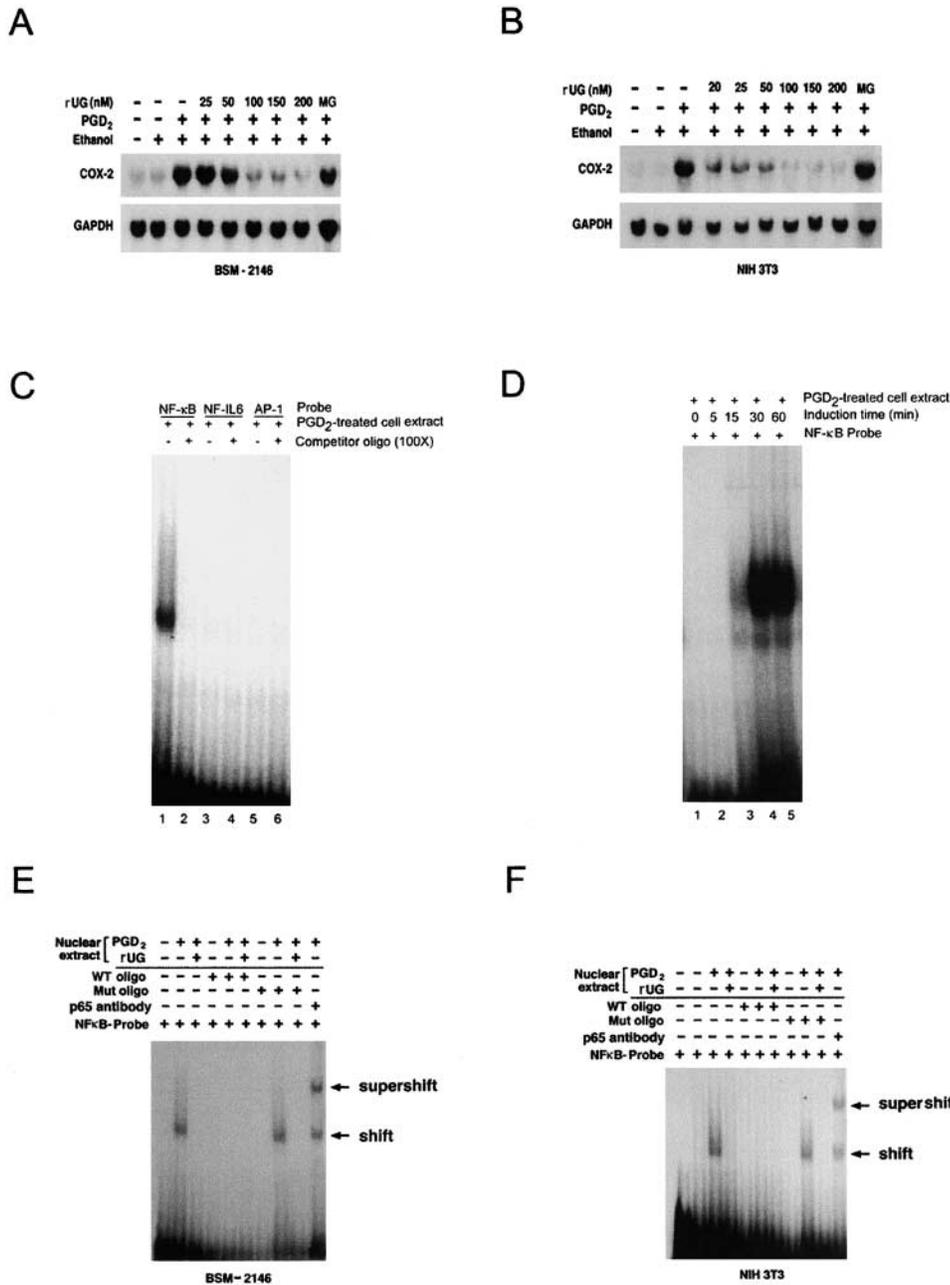


Figure 6. UG inhibits DP-mediated NF-κB activation and COX-2 expression. Expression of COX-2 mRNA in BSM 2146 (A) and NIH-3T3 cells (B). Cells were treated with PGD₂ in the presence of rUG (0–200 nM) or MG (200 nM). Note that rUG inhibits DP-mediated COX-2 mRNA expression in a dose-dependent manner in both BSM-2146 (A) and NIH-3T3 (B), whereas MG has no such inhibitory effects (A and B, last lanes). EMSA using ³²P-labeled double stranded NF-κB, NF-IL6, and AP-1 oligo probes in the absence and presence of respective double stranded competitor unlabeled oligo, demonstrating the effect of DP signaling on the activation of NF-κB in NIH-3T3 (C) cells. This activation of NF-κB by PGD₂ is time dependent (D). EMSA demonstrating the effect of DP signaling on the activation of NF-κB in BSM-2146 (E) and NIH-3T3 (F) cells treated with PGD₂ in the absence or presence of 150 nM purified rUG. Note rUG treatment before the addition of PGD₂ inhibits NF-κB activation in both cell types.

Discussion

Allergen-induced hypersensitivity and inflammation represent a growing public health problem in the industrialized world, affecting nearly 30% of the population <30 yr of age (31). The molecular mechanisms that underlie the pathophysiology of these conditions are poorly understood. In the present study, we uncovered several new and important aspects of allergen-induced inflammatory response and a possible innate homeostatic mechanism that represses such responses. More specifically, we found that: (a) in response to allergen (OVA) sensitization and challenge, UG-KO mice manifest significantly elevated levels of COX-2 gene expression in the lungs compared with that of the WT littermates; (b) in response to

OVA sensitization and challenge, these mice also express elevated levels of eotaxin and Th2 cytokines, such as IL-4, IL-5, and IL-13, manifesting a markedly increased eosinophil infiltration in the lungs; and (c) most importantly, rUG treatment of the OVA-sensitized UG-KO mice before OVA challenge abrogates these allergic inflammatory responses. Using an in vitro system, we further demonstrate that (a) PGD₂ mediates COX-2 expression specifically via its receptor, DP, and (b) DP signaling is mediated via p38 MAPK, p44/42 MAPK, or PKC in a cell type-specific manner activating NF-κB required for the expression of COX-2, an agonist-inducible enzyme critical for the production of inflammatory lipid me-

diators. Further, rUG treatment of the cells blocks PGD₂-stimulated NF- κ B activation and COX-2 expression.

PGD₂ is an inflammatory eicosanoid released into human airways upon acute antigen challenge (7). Moreover, human lung and mast cells have been reported to generate PGD₂ in a calcium-dependent manner (9, 11). Our results show a marked reduction of PGD₂ levels in the BALF of OVA-sensitized UG-KO mice that are treated with rUG before OVA challenge. Previous reports indicate that UG is a potent antichemotactic agent for monocytes and neutrophils (32, 33). Thus, UG is likely to have similar antichemotactic effects on eosinophils and mast cells preventing lung infiltration. Since activated mast cells secrete PGD₂, rUG may reduce the level of this eicosanoid in the lungs of allergen-challenged UG-KO mice by inhibiting the migration of mast cells. Recently, using a mouse model of allergic airway inflammation Honda et al. (13) have reported that inhalation of aerosolized PGD₂ by OVA-sensitized mice precipitated allergic inflammatory response in the lungs. Since acute antigen challenge stimulates PGD₂ release (7) and this prostanoid is a potent chemoattractant for human eosinophils (24), it is likely that UG gene is strategically expressed in the airway epithelia to protect this vital organ from allergen-induced, PGD₂-mediated eosinophil infiltration.

Although it has been reported that PGD₂ mediates allergic inflammation via its receptor, DP, the molecular mechanism(s) of DP signaling and how it mediates inflammatory response, until now, remained unclear. Since one of the major goals of this study was to understand the mechanism(s) by which UG prevents the allergen-induced inflammatory responses, we determined whether UG plays any role in down-regulating DP signaling and PGD₂-stimulated COX-2 expression. We first uncovered that DP signaling is mediated via p38 MAPK, P44/42 MAPK, and PKC in a cell type-specific manner leading to the activation of NF- κ B and subsequent stimulation of COX-2 gene expression. To our knowledge, this is the first report delineating the mechanism of DP-mediated activation of NF- κ B and consequent stimulation of COX-2 gene expression in three different cell types. Most importantly, our results show that rUG inhibits DP-mediated NF- κ B activation and OVA-induced stimulation of PGD₂ levels in BALF. In addition, rUG inhibits OVA-induced expression of eotaxin, several Th2 cytokines (i.e., IL-4, IL-5, IL-13), and COX-2, a critical enzyme for the production of inflammatory lipid mediators. Cytokines such as IL-4, IL-5, and IL-13 are capable of inducing IgE production and activation of mast cells and eosinophils (34, 35). Our results show that the level of these cytokines is markedly reduced by rUG treatment of OVA-sensitized UG-KO mice before OVA challenge. IL-13 has been suggested to play a critical regulatory role in mediating allergic inflammation (36–38) and has been reported to stimulate UG gene expression in rat lungs (23). Therefore, UG may have a homeostatic role in down-regulating allergic inflammatory response. Our results indicate that UG suppresses allergic inflammatory response not only by inhibiting COX-2 expression and reduction of

PGD₂ levels in the BALF but also by inhibiting the production of proinflammatory cytokines and eotaxin.

Structural studies have delineated that UG is a homodimeric protein in which the identical 70-amino acid subunits are joined in antiparallel orientation by two inter-chain disulfide bonds forming a central hydrophobic cavity (3, 4). Previous reports have indicated that UG binds hydrophobic ligands such as progesterone (39) and retinol (40), raising the possibility that it may also bind and possibly sequester other hydrophobic ligands such as PGD₂. We have conducted molecular modeling studies using the crystallographic coordinates of human rUG (24), and we have shown that the structural features of UG readily allow docking of PGD₂ into its central hydrophobic cavity (Supplemental Materials and Methods and Fig. S2, available at <http://www.jem.org/cgi/content/full/jem.20031666/DC1>). Consistent with this finding, the results of our experiment show that when ³H-PGD₂ is incubated with rUG a radioactive PGD₂-UG complex is readily detectable by SDS-PAGE analysis (Supplemental Materials and Methods and Fig. S3, available at <http://www.jem.org/cgi/content/full/jem.20031666/DC1>). Thus, it appears that at least one of the mechanisms by which UG may prevent DP-mediated NF- κ B activation and COX-2 gene expression is by sequestering this eicosanoid into its hydrophobic cavity, thereby suppressing the inflammatory response. Since UG is one of the most abundant proteins constitutively expressed in the lungs (μ g/ml of BALF) and our results indicate that the maximum PGD₂ levels in the BALF of OVA-sensitized and -challenged WT mice are pg/ml range, the stoichiometry appears to favor our proposed model.

PGD₂ is a potent chemoattractant for eosinophils (24), and transgenic mice in which PGD₂ synthase overexpression is targeted to the lungs manifest elevated Th2 cytokines and eosinophil infiltration (14). Recently, it has been reported that aerosolized PGD₂ reinforces allergen-induced inflammatory response via macrophage-derived chemokines (13). Although our results reconfirm the important role of PGD₂ in allergen-induced inflammatory response and the critical role of UG in suppressing such response mediated via DP signaling, these results also suggest that UG suppresses the production of OVA-induced chemokines. Our results also demonstrate that UG inhibits OVA-induced stimulation of eosinophil infiltration in the lungs of UG-KO mice. This observation is consistent with our previous finding that UG inhibits chemotaxis of monocytes and neutrophils (32, 33), although the molecular mechanism(s) of this effect of UG is not yet clear. Since UG binds to cell surface binding proteins (putative receptors) (41), future studies may delineate whether the antichemotactic effects of UG are mediated via these binding proteins.

It has been reported that although UG gene expression in the airway epithelia is constitutive, its expression is further augmented by glucocorticoids (42), the most effective drug currently available for the treatment of airway inflammation. Although the administration of nonsteroidal anti-inflammatory drugs, which are known to inhibit both

COX-1 and COX-2 enzymes (16), may have catastrophic consequences in ~10% of the adults with asthma, it has been reported that selective COX-2 inhibitors such as rofecoxib and celecoxib do not manifest such adverse reactions (43, 44). Thus, the commonly available nonsteroidal anti-inflammatory drugs may have effects in addition to the COX-2 inhibitory activity that causes the adverse complications in asthmatic patients. On the other hand, glucocorticoids, which inhibit COX-2 gene expression and reduce prostaglandin levels (45), remain the mainstay of current treatment for airway inflammation in asthma, which often has an allergic component. Although the mechanism(s) of antiinflammatory effects of glucocorticoids is still the subject of intense investigation, a recent study has elegantly demonstrated that targeted disruption of a glucocorticoid-inducible gene, lipocortin-1 (annexin-1) (46), in mice causes increased COX-2 expression and manifests an exaggerated inflammatory response (47). These results are consistent with those observed in our UG-KO mice.

We have reported previously that UG-deficient mice manifest abnormal deposition of fibronectin and IgA in the kidney glomeruli (48), reminiscent of human IgA nephropathy. Further, the results of our present investigation together with those of our previous study (6) indicate that UG-KO mice are susceptible to developing exaggerated inflammatory response to allergen challenge that is abrogated by rUG treatment. This may explain a long-standing unexplained linkage between inflammation in the respiratory system with disease pathology in the renal system. Several reports have documented that inflammatory lung diseases are associated with abnormal deposition of IgA and extracellular matrix proteins, such as fibronectin, in the renal glomeruli (49–53) as we have reported in UG-KO mice (5). These observations, together with the facts that (a) chronic airway inflammation markedly suppresses UG production (53) and (b) the UG-KO mice (5) develop IgA nephropathy (48) may further support the notion that pulmonary inflammation and renal pathologies are causally related. Our results demonstrate the importance of UG and perhaps of other proteins with similar properties in maintaining homeostasis in two different vital organs, the lungs and the kidneys.

The respiratory system and the renal system communicate with the external environment providing a portal of entry for microorganisms and other foreign antigens and allergens. During the last decade, tremendous progress has been made in delineating the innate defense mechanisms that exist against microbial pathogens (54). However, even after the pathogens are destroyed by the innate microbial defense system, the respiratory system must also cope with inadvertent stimulation of inflammation induced by antigens associated with these pathogens. The mechanism(s) by which the majority of the individuals avert inadvertent stimulation of inflammatory response despite constant exposure to foreign antigens remains a mystery. It is tempting to speculate that UG is strategically expressed in organs that communicate with the external environment and provide protection

against allergen-induced stimulation of the inflammatory response. Further understanding of the molecular mechanisms of action of UG and other related proteins may allow the development of novel therapeutic approaches to at least some allergic and inflammatory disorders.

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