Induction of Cyclooxygenase-2 Expression in Glomeruli by Aggregated Protein

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ABSTRACT—Cyclooxygenase has two isozymes, a constitutive type (cyclooxygenase-1) and an inducible type (cyclooxygenase-2). The aim of the present study was to determine whether cyclooxygenase -2 is associated with the increased production in prostaglandin E_2 in glomeruli by aggregated protein. Mice were injected with aggregated bovine serum albumin. Glomeruli were isolated using sieves and a magnet. Production of prostaglandin E_2 was increased in glomeruli after injection of aggregated bovine serum albumin. RT-PCR analysis indicated enhanced expression of cyclooxygenase-2 mRNA in aggregated bovine serum albumin-loaded glomeruli. Western blotting analysis indicated an increase in cyclooxygenase-2 protein in glomeruli by aggregated bovine serum albumin. Glomeruli were incubated with indomethacin, NS-398 or niflumic acid in the presence of arachidonic acid. Indomethacin resulted in remarkable reduction of prostaglandin E_2 levels in aggregated bovine serum albumin-loaded glomeruli. Niflumic acid also inhibited prostaglandin E_2 production, and its inhibitory rate was more than that of NS-398. In conclusion, aggregated protein induces cyclooxygenase-2 in glomeruli, suggesting that cyclooxygenase-2 is involved in the process of disposal of aggregated protein in glomeuli.

Keywords: Cyclooxygenase-2, Aggregated protein, Glomeruli, NS-398, Niflumic acid

Arachidonic acid is generated from cell membrane phospholipid by phospholipase A_2, and is converted to prostaglandin E_2 by the action of cyclooxygenase and prostaglandin E synthase. Two isozymes of cyclooxygenase have been cloned, cyclooxygenase-1 and cyclooxygenase-2 (1). These molecules have approximately 60% homology at the amino acid level. Cyclooxygenase-1 is constitutively expressed in various tissues. In particular, prostaglandin E_2 produced by cyclooxygenase-1 plays an important role in maintenance of blood flow in the kidney and the stomach. On the other hand, cyclooxygenase-2 is an inducible enzyme and is induced by inflammation and stimulation by proinflammatory cytokines (2). Interleukin-1β induces expression of cyclooxygenase-2 mRNA in rat mesangial cells (3), and expression of cyclooxygenase-2 is regulated by glucocorticoids (4). Recently, cyclooxygenase-2 was reported to be constitutively expressed in the kidney and is considered to play important roles in renin release to regulate sodium balance and renal blood flow (5, 6). The differences in expression between cyclooxygenase-1 and -2 are considered to be due to differences in the promoters of each gene. The promoter of cyclooxygenase-1 has binding sites for specificity protein 1 (SP-1) and activator protein 2 (AP-2), but not a TATA box. On the other hand, the promoter of cyclooxygenase-2 contains inducible transcription factor binding sites such as those for nuclear factor-interleukin-6 (NF-IL-6) and nuclear factor-κB (NF-κB) (7, 8). The cyclooxygenase-2 promoter also has a cyclic adenosine 5'-monophosphate response element (CRE) and TATA box (9, 10).

Previously, we reported that aggregated bovine serum albumin was accumulated in glomeruli and then removed from glomeruli by 48 h in mice (11). The prostaglandin E_2 level was increased in aggregated bovine serum albumin-loaded glomeruli-rich fraction, but prostaglandin E_2 was not increased by 9 h after injection of aggregated bovine serum albumin. Therefore, we considered that cyclooxygenase-2 would be induced in glomeruli by aggregated bovine serum albumin. In this study, we isolated mouse glomeruli with high purity and examined whether cyclooxygenase-2 expression is the cause of the enhanced production of prostaglandin E_2 in aggregated bovine serum albumin-loaded glomeruli. We demonstrated that aggregated

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protein enhances expression of cyclooxygenase-2 in glomeruli.

MATERIALS AND METHODS

Animals

Male ICR mice, weighing approximately 25 g, were obtained from Charles River (Yokohama). Mice were housed in an air-conditioned room at $22 \pm 2°C$ under a 12-h light-dark cycle until the experiments. Animals had free access to water and standard mouse chow.

Reagents

Drugs were purchased from the following sources: crystallized bovine serum albumin was from Serologicals Proteins, Inc. (Kankakee, IL, USA); iron oxide was from Aldrich Chemical Company (Milwaukee, WI, USA); collagenase type II and deoxyribonuclease I were from Sigma Chemical (St. Louis, MO, USA); AmpliTaq Gold DNA polymerase was from Roche Molecular Systems, Inc. (Branchburg, NJ, USA); TaqMan Gold RT-PCR Kit, TaqMan rodent glyceraldehyde-3-phosphate dehydrogenase control reagents, and Super Script II were from Life Technologies, Inc. (Grand Island, NY, USA). The following reagents and materials were also used: protease inhibitor cocktail (Sigma Chemical); Nonidet NP-40 (Amresco, Solon, OH, USA); Ready Gel J 7.5%, horseradish peroxidase-conjugated anti-immunoglobulin G, and horseradish peroxidase-conjugated streptavidin (Bio-Rad Laboratories, Richimond, CA, USA); Hybond-C pure nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA); polyclonal antibody to mouse cyclooxygenase-1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); polyclonal antibody to mouse cyclooxygenase-2 (Takara Syuzo, Tokyo); horseradish peroxidase-conjugated antibody to immunoglobulin G for cyclooxygenase-1 (Chemicon International, Inc., Temecula, CA, USA); horseradish peroxidase-conjugated antibody to immunoglobulin G for cyclooxygenase-2 (Promega, Madison, WI, USA); indomethacin, arachidonic acid and dimethyl sulfoxide (Wako Pure Chemical Industries, Osaka); N-2-(cyclohexyloxy-4-nitrophenyl) methane sulfonamide (NS-398), 2[[3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid (niflumic acid) and prostaglandin E_2 (Cayman Chemical, Ann Arbor, MI, USA). NS-398 and niflumic acid were dissolved in dimethyl sulfoxide and diluted in Krebs-Ringer phosphate buffer. Arachidonic acid was dissolved in ethanol and diluted in Krebs-Ringer phosphate buffer.

Time course of prostaglandin E_2 production in glomeruli by aggregated protein

Aggregated bovine serum albumin was prepared according to the previously reported procedure (11). Mice were injected i.v. with 0.8 mg/g body weight of aggregated bovine serum albumin at 0 and 3 h in this and following experiments. To determine the time course of prostaglandin E_2 production, glomeruli were isolated by the procedure of Assmann et al. (12) with some modifications at 0, 3, 8 and 24 h after the first injection of aggregated bovine serum albumin. Briefly, kidneys were perfused with 1 mg/ml of iron oxide in phosphate-buffered saline and removed. The kidneys were pressed through a 90-μm mesh screen and collected on a 38-μm mesh screen. Glomeruli with iron oxide were isolated with a magnet (Magical Trapper; Toyobo, Osaka) from the suspension of glomeruli and renal tubules. The purity of glomeruli was over 95% as observed by light microscopy. Isolated glomeruli were incubated for 10 min at room temperature in Krebs-Ringer phosphate buffer containing deoxyribonuclease I (25 units/ml) and collagenase type II (0.01 mg/ml) for permeabilization (13). Glomeruli (2,500/0.5 ml) were suspended in fresh Krebs-Ringer phosphate buffer and incubated in a CO_2 incubator for 60 min. The incubation medium was obtained by centrifugation at $34.9 \times g$ for 5 min at 4°C and stored at −80°C with 10 mg/ml of indomethacin. Prostaglandin E_2 was determined using an enzyme immunoassay kit (Amersham Pharmacia Biotech UK Limited).

RNA isolation and RT-PCR analysis for cyclooxygenase-2 mRNA

Semi-quantitative RT-PCR analysis: The glomeruli were isolated 0, 1, 3, 6, 8 and 24 h after the first injection of aggregated bovine serum albumin. Total RNA was extracted from glomeruli using a Fast RNA Green Kit (BIO 101 Systems, La Jolla, CA, USA) and Fast Prep FP 120 (Savant Instrument Inc., Holbrook, NY, USA). Reverse transcription was performed with Super-script II according to the manufacturer's protocol. The resulting cDNA was amplified using a TaKaRa PCR Thermal Cycler (Takara Syuzo). cDNA samples were subjected to 36 cycles of heating at 94°C for 15 s followed by 60°C for 30 s for cyclooxygenase-2, or 36 cycles of 94°C for 45 s and 65°C for 45 s for glyceraldehyde-3-phosphate dehydrogenase. The sequences of primers were as follows: cyclooxygenase-2 sense primer, 5'-TGA CCC CCA AGG CTC AAA TAT-3', antisense primer, 3'-CCC AGG TCC TCG CTT ATG ATC T-5'; glyceraldehyde-3-phosphate dehydrogenase sense primer, 5'-AGC CGC ATC TTC TTG TGC A-3', antisense primer, 3'-GCC GTG AGT GGA GTC ATA CT-5'. PCR products were analyzed by electrophoresis on 3% agarose gels. Ethidium bromide-stained bands were analyzed with NIH image.

Real-time RT-PCR: Renal RNA of mice injected with lipopolysaccharide (0.3 mg/mouse, i.v.) was used as a control for cyclooxygenase-2. An ABI PRISM 7700 Sequence Detection System (PE Applied Biosystem, Tokyo) was

used for this analysis. Reverse transcription was performed with the control RNA and the sample in MicroAmp 96-well Optical Reaction Plates (PE Applied Biosystem) for 10 min at 25°C, for 30 min at 48°C and for 5 min at 95°C. PCR was performed using TaqMan PCR reagent, Ampli Taq Gold, and the primers described above for 40 cycles of 2 min at 50°C, for 30 min at 48°C, for 5 min at 95°C, for 15 s at 95°C, and for 60 s at 60°C. After PCR, the amplification curves of cyclooxygenase-2 and glyceraldehyde-3-phosphate dehydrogenase mRNAs were obtained. Then the amount of cyclooxygenase-2 mRNA was determined from the standard curve prepared from control mRNA.

Western blotting analysis of cyclooxygenase-1 and cyclooxygenase-2

Glomeruli were isolated 0, 8, 16 and 24 h after the first injection of aggregated bovine serum albumin. Glomeruli (20,000/ml) were suspended in lysis buffer (300 mM NaCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 0.2 mM Tris) including NP-40 and protease inhibitor cocktail. Glomeruli were disrupted twice by ultrasonication for 15 min and then centrifuged at $(1.26 \times 10^4) \times g$ for 10 min at 4°C. Proteins were measured by the Lowry procedure (DC Protein Assay kit; Bio-Rad Laboratories) in the supernatant. The supernatant (3 μg protein) was dissolved in Laemmli sample buffer (50 mM Tris-HCl pH 6.8, 2% sodium dodecyl sulfate, 6% β-mercaptoethanol, bromophenol blue), heated at 95°C for 4 min, and cooled to room temperature. Proteins were then separated by electrophoresis on a 7.5% SDS polyacrylamide gel. The separated proteins were then transferred onto Hybond-C pure nitrocellulose membranes. Nonspecific binding was blocked with 5% skim milk in phosphate-buffered saline solution. The membranes were incubated for 1 h with anti-mouse cyclooxygenase-1 polyclonal antibody or anti-mouse cyclooxygenase-2 polyclonal antibody. The membranes were incubated with horseradish peroxidase-conjugated anti-immunoglobulin G or horseradish peroxidase-conjugated streptavidin in phosphate-buffered saline plus Tween 20 solution. Then, the membranes were reacted with ECL Western blotting detection reagents (Amersham Pharmacia Biotech) and photographed on hyperfilm ECL (Amersham Pharmacia Biotech).

Pharmacological manipulation of prostaglandin E2 production in glomeruli

Mice were injected twice with aggregated bovine serum albumin. Glomeruli were isolated 8 h after the first injection and permeabilized. Glomeruli were incubated with vehicle, indomethacin ($10^{-5} - 10^{-7}$ M), NS-398 ($10^{-5} - 10^{-7}$ M) or niflumic acid ($10^{-5} - 10^{-7}$ M) for 60 min in a CO_2 incubator in the presence of arachidonic acid (30 μM). Prostaglandin E_2 in the incubation medium was determined

as described above.

Statistical analyses

Results are expressed as means ± S.E.M. Statistical analyses of data were carried out with Student's t-test or one-way analysis of variance (ANOVA). To determine the significance of differences among the groups, the Bonferroni procedure was used. Differences between the mean values of the experimental and control groups were considered significant at $P<0.05$.

RESULTS

Time course of prostaglandin E2 production in glomeruli

As shown in Fig. 1, there was no significant increase in prostaglandin E_2 production by aggregated bovine serum albumin 3 h after the first injection of aggregated bovine serum albumin as compared with that in control glomeruli. When glomeruli were isolated 8 h after the first injection, namely at 5 h after the second injection, aggregated bovine serum albumin-loaded glomeruli showed 53% more prostaglandin E_2 than controls. Prostaglandin E_2 level then decreased, but it was still significantly higher than that in controls after 24 h.

Detection of cyclooxygenase-2 mRNA by RT-PCR analysis

We investigated the expression of cyclooxygenase-2 mRNA in glomeruli using an RT-PCR-based method. On
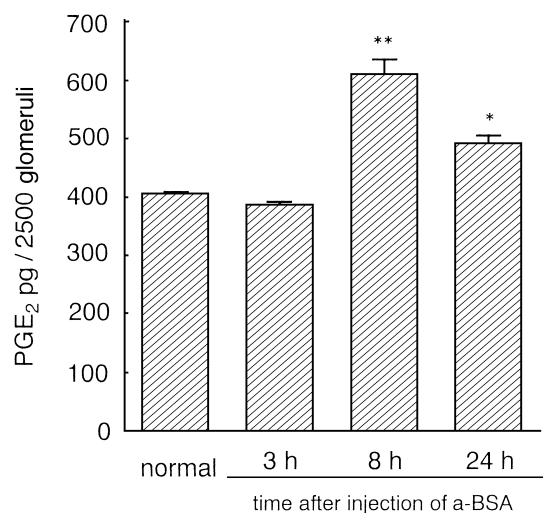

Fig. 1. Prostaglandin E_2 (PGE_2) production of glomeruli in mice after aggregated bovine serum albumin (a-BSA) injection. Mice were injected with a-BSA at 0 and 3 h. Glomeruli were isolated 0, 3, 8 and 24 h after the first injection of a-BSA and incubated for 60 min at 37°C. Normal controls (0-h group) received no a-BSA injection. The second isolation of glomeruli (3-h group) was performed before the second injection of a-BSA, and 8-h- and 24-h-group received twice injection of a-BSA. Results are expressed as means ± S.E.M. of 5 mice. *$P<0.05$, **$P<0.01$, compared with the normal controls.

COX-2 mRNA 89 b.p.→

GAPDH mRNA 200 b.p.→

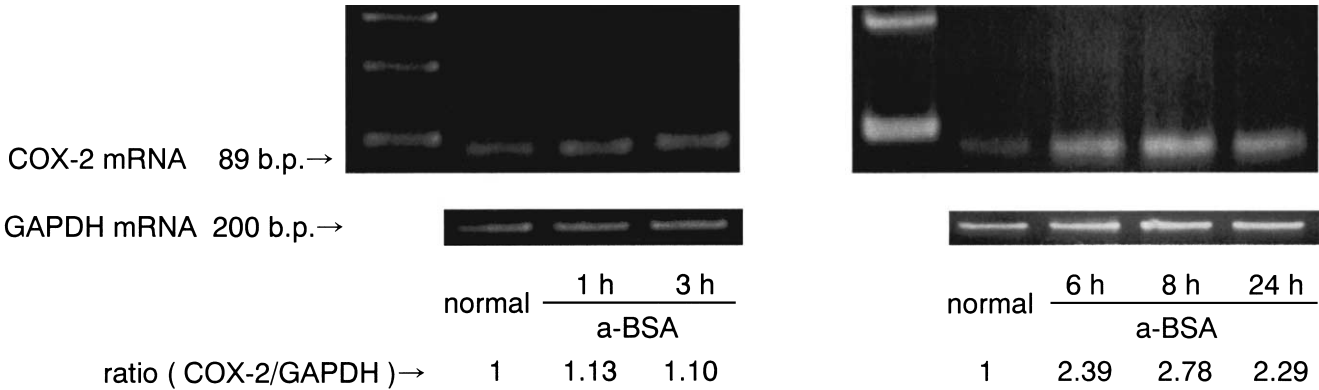|	normal	1 h	3 h		normal	6 h	8 h	24 h
		a-BSA				a-BSA		
ratio (COX-2/GAPDH)→	1	1.13	1.10		1	2.39	2.78	2.29

Fig. 2. Semi-quantitative RT-PCR analysis of cyclooxygenase-2 (COX-2) mRNA expression in glomeruli. Mice were injected with aggregated bovine serum albumin (a-BSA) at 0 and 3 h. Glomeruli were isolated 0, 1, 3, 6, 8 and 24 h after the first injection of a-BSAand total RNA was isolated from 4 mice. Normal group received no a-BSA injection; 1-h and 3-h-groups received once injection of a-BSA; and the 6-h, 8-h and 24-h-groups received two injections of a-BSA.

semi-quantitative RT-PCR analysis, cyclooxygenase-2 mRNA expression was increased by threefold over that in glomeruli at 8 h by two injections of aggregated bovine serum albumin, and this increase continued until 24 h (Fig. 2). Obvious increase was not observed at 1 h and 3 h in the glomeruli that were obtained from mice subjected to one injection of aggregated bovine serum albumin. We further quantitatively analyzed using real-time RT-PCR whether cyclooxygenase-2 mRNA expression was increased by aggregated bovine serum albumin in the glomeruli obtained from mice that received two injections of aggregated bovine serum albumin. Cyclooxygenase-2 mRNA levels were significantly increased by 25% by aggregated bovine serum albumin as compared to those in the control glomeruli at 8 h (Fig. 3).
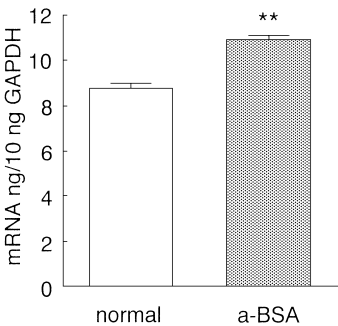
Detection of cyclooxygenase-2 protein by Western blot analysis

We analyzed cyclooxygenase-2 protein levels in glomeruli that were isolated 8, 16 and 24 h after injection of aggregated bovine serum albumin. Cyclooxygenase-2 protein expression in aggregated bovine serum albumin loaded-glomeruli were increased 8 h after the injection as compared with those in the control glomeruli (Fig. 4). They were also distinguished from the control glomeruli 16 and 24 h after the injection. Cyclooxygenase-1 protein was also detected in glomeruli. There was no difference in cyclooxygenase-1 protein expression in glomeruli as compared with the controls (data not shown).

Effects of cyclooxygenase inhibitor on prostaglandin E_2 production

We performed pharmacological manipulations to clarify the involvement of cyclooxygenase-2 in increased renal

Fig. 3. Real time RT-PCR analysis of cyclooxygenase-2 mRNA expression in glomeruli. Mice were injected with aggregated bovine serum albumin (a-BSA) at 0 and 3 h. Glomeruli were isolated 8 h after the first injection of a-BSA and total RNA was isolated from 4 mice. Results are expressed as means ± S.E.M. of 3 experiments. **$P<0.01$, compared with the normal controls that received no a-BSA injection.

COX-2
72 kD
→
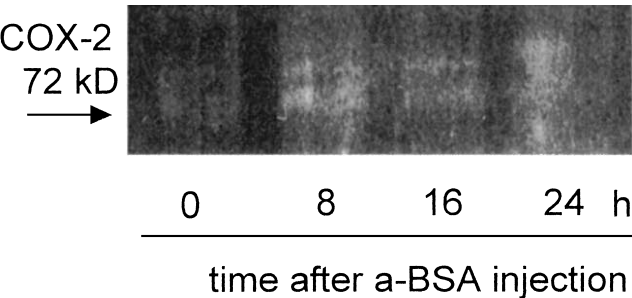
0   8   16   24   h

time after a-BSA injection

Fig. 4. Western blotting analysis of cyclooxygenase-2 (COX-2) in glomeruli. Mice were injected with aggregated bovine serum albumin (a-BSA) at 0 and 3 h. Glomeruli were isolated each hour after the first injection of a-BSA and protein was extracted from 20,000 glomeruli. Samples (3 μg protein, each) were applied to an SDS polyacrylamide gel.
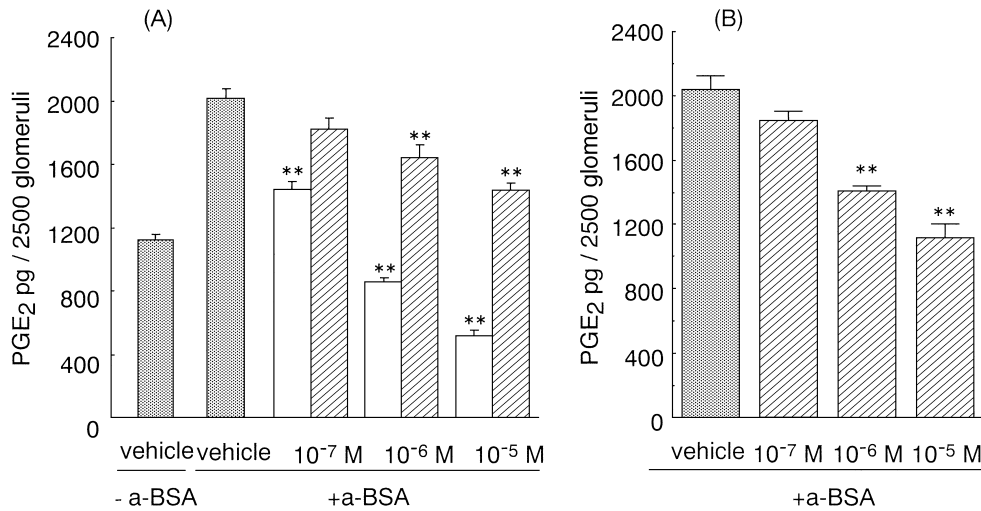
Fig. 5. Effects of cyclooxygenase inhibitors on prostaglandin E_2 (PGE_2) production of glomeruli. Mice were injected twice with aggregated bovine serum albumin (a-BSA) at 0 and 3 h. Glomeruli were isolated 8 h after the first injection of a-BSA. Glomeruli were incubated with vehicle or cyclooxygenase inhibitor in the presence of 30 μM arachidonic acid. Open column shows the indomethacin-treated group. Striped columns indicate the NS-398-treated group (A) and the niflumic acid-treated group (B). Stippled columns indicate vehicle-treated controls. Results are expressed as means ± S.E.M. of 4 mice (A) and 7 mice (B). **$P<0.01$, compared with the vehicle group.

production of prostaglandin E_2 by aggregated bovine serum albumin. Glomeruli were incubated with indomethacin, NS-398, or niflumic acid in the presence of arachidonic acid. Indomethacin resulted in marked suppression of prostaglandin E_2 production (75% at 10^{-5} M) in aggregated bovine serum albumin-loaded glomeruli in a dose-dependent manner (Fig. 5). The levels of prostaglandin E_2 were less than those in vehicle-treated normal glomeruli ($-$BSA). NS-398 (at 10^{-5} M) inhibited the increase in prostaglandin E_2 level in aggregated bovine serum albumin-loaded glomeruli to a level 29% less than that in the vehicle control group ($+$BSA), and niflumic acid decreased it to a level 45% less than the control at 10^{-5} M (Fig. 5).

DISCUSSION

In the present study, we demonstrated that 1) prostaglandin E_2 production was increased in mouse glomeruli by aggregated bovine serum albumin, 2) aggregated bovine serum albumin promoted expression of cyclooxygenase-2 mRNA and cyclooxygenase-2 protein, and 3) the selective cyclooxygenase-2 inhibitors interfered with the increase in production of prostaglandin E_2.

Cyclooxygenase-2 has been considered to be an inducible, and not a constitutive, enzyme ($1-4$). Cyclooxygenase-2, however, is constitutively expressed as well as cyclooxygenase-1 in the kidney, which generates high levels of prostaglandins. The localization of cyclooxygenase-2 was immunohistochemically demonstrated in the cortical collecting duct in the mouse kidney (14). In the rat kidney,

cyclooxygenase-2 message and protein were demonstrated in the macula densa and cortical thick ascending limb (5). Although many investigators have reported consistent observations concerning the localization of cyclooxygenase-1 in glomeruli, that of cyclooxygenase-2 in glomeruli has been controversial. Cyclooxygenase-2 mRNA was occasionally contained at low levels in glomeruli in rats (6). Interestingly, cultured mesangial cells also express low levels of cyclooxygenase-2 mRNA (6). Moreover, immunoreactive cyclooxygenase-2 protein was distinctly demonstrated in glomerular epithelial cells in human adult kidneys and at the end stage of renal development in fetal human kidneys (15). We isolated glomeruli with high purity (>95%) and detected cyclooxygenase-2 in glomeruli by RT-PCR analyses and Western blotting (Figs. 2 and 4). Therefore, it is likely that cyclooxygenase-2 is expressed in mouse glomeruli, although it remains possible that cyclooxygenase-2 may be induced during the isolation of glomeruli.

In the present study, we confirmed additional production of prostaglandin E_2 (Fig. 1), and demonstrated increase in cyclooxygenase-2 mRNA in aggregated bovine serum albumin-loaded glomeruli (Figs. 2 and 3). These changes were induced several hours after two injections of aggregated bovine serum albumin. We did not observe an increase in prostaglandin E_2 and cyclooxygenase-2 mRNA in glomeruli isolated from mice that were injected once with aggregated bovine serum albumin. It is speculated that aggregated bovine serum albumin of one injection may be insufficient to stimulate mesangial cells in glomeruli. In

experimental models of glomerulonephritis, there have been reports regarding increases in prostaglandin E_2 and up-regulation of cyclooxigenase-2 mRNA in the glomeruli. Expression of cyclooxygenase-2 mRNA is enhanced in glomeruli in anti-Thy-1 glomerulonephritis, which is a model of mesangial proliferative glomerulonephritis (16). Furthermore, complement-mediated glomerular injury, which is a model of membranous glomerulonephritis, is associated with up-regulation of cyclooxygenase-2 mRNA (17). In addition, in cultured mesangial cells, cyclooxygenase-2 mRNA is strongly induced by proinflammatory cytokines (18). As aggregated bovine serum albumin is accumulated in glomeruli and is localized to the mesangial area and epithelial cells (11), aggregated proteins may activate mesangial cells and/or epithelial cells. Therefore, it is reasonable to consider that glomeruli enhance expression of cyclooxygenase-2 mRNA by aggregated protein. On the other hand, expression of cyclooxygenase-2 mRNA varies not only in pathological glomeruli but also in the cortical thick ascending limb of rat kidney with salt intake or salt restriction (5, 6). We do not exclude the possibility that the presense of a fraction of renal tubules and macula densa might influence the present findings of aggregated protein-loaded glomeruli regarding expression of cyclooxygenase-2 mRNA.

As we confirmed that cyclooxygenase-2 mRNA level was significantly increased in aggregated bovine serum albumin-loaded glomeruli by real-time RT-PCR, we performed experiments to clarify the increase in cyclooxygenase-2 protein level in the aggregated bovine serum albumin-loaded glomeruli. We demonstrated an increase in cyclooxygenase-2 protein (Fig. 4). In the present experiments using real-time RT-PCR, we demonstrated a 25% increase in cyclooxygenase-2 mRNA level in response to two injections of aggregated protein (Fig. 3). This moderate increase in expression of mRNA was enough to produce differences in protein level. We also examined the increase in cyclooxygenase-2 expression by pharmacological manipulation of aggregated bovine serum albumin-loaded glomeruli, using indomethacin, a cyclooxygenase-1 and -2 inhibitor, and NS-398 and niflumic acid, selective cyclooxygenase-2 inhibitors. NS-398 markedly attenuated production of prostaglandin E_2 in a mouse collecting duct cell line in which cyclooxygenase-2 is abundantly expressed (14). The concentration of NS-398 in that previous report (14) was the same as the highest concentration in the present study. Production of additional prostaglandin E_2 was inhibited by coincubation with NS-398 in a concentration-dependent manner (Fig. 5). Indomethacin markedly suppressed prostaglandin E_2 production to lower levels than those in normal controls, indicating that both cyclooxygenases were inhibited by indomethacin (Fig. 5). NS-398 exerted moderate inhibitory effects on prostaglandin E_2

production in aggregated bovine serum albumin-loaded glomeruli. Another selective cyclooxygenase-2 inhibitor, niflumic acid, inhibited production of additional prostaglandin E_2 as well as NS-398. The IC_{50} for human recombinant cyclooxygenase-1 and cyclooxygenase-2 is 75 and 1.77 μM with NS-398, and 16 and 0.1 μM with niflumic acid, respectively (19). The stronger inhibitory effect was observed by using niflumic acid in isolated glomeruli. Although we expected remarkable suppression of prostaglandin E_2 production in isolated glomeruli since cyclooxygenase-2 protein was induced by aggregated bovine serum albumin, cyclooxygenase-2 inhibitors showed less effect as compared with that of indomethacin. The present pharmacological manipulation test was performed in the presence of arachidonic acid (30 μM). A large amount of arachidonic acid (>2.5 μM) is efficiently converted by cyclooxygenase-1 in NIH 3T3 cells (20). In addition, in fibroblasts derived from cyclooxygenase-2-deficient mice, cyclooxygenase-1 partially makes up for the missing function of cyclooxygenase-2 (21). Many investigators have demonstrated that glomeruli primarily express cyclooxygenase-1. Therefore, we speculated that glomeruli vigorously convert exogenous arachidonic acid to prostaglandin E_2 under conditions in which cyclooxygenase-2 activity is suppressed. Based on these findings, it is reasonable to conclude that the enhanced production of prostaglandin E_2 was the result of de novo cyclooxygenase-2 production induced by aggregated bovine serum albumin.

These findings raise the question of why production of prostaglandin E_2 was increased in glomeruli by deposition of aggregated protein. We reported previously that prostaglandin E_2 accelerates the process of disposal of aggregated protein in glomeruli and in cultured mesangial cells (22, 23). As shown in the present study, an increase in cyclooxygenase-2 expression is, therefore, required to produce prostaglandin E_2 and accelerate removal of aggregated protein from glomeruli. Cyclooxygenase-2 knock-out mice have been shown to develop glomerular hypertrophy and glomerular sclerosis (24). In patients with chronic glomerulonephritis or glomerulosclerosis, large amounts of protein are deposited in the glomeruli (25, 26). Repeated administration of bovine serum albumin causes glomerular injury via increases in levels of intraglomerular platelet-derived growth factor-B and interleukin-1β mRNA expression in mice (27). The long-term deposition of macromolecules in glomeruli is considered to cause inflammation and glomerular injury, which can lead to dysfunction of the kidneys (28). Therefore, cyclooxygenase-2 seems to play critical roles in processing of aggregated protein in glomeruli.

Further investigations are required to clarify the mechanisms responsible for induction of cyclooxygenase-2 expression in glomeruli.

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