

Indomethacin protects permeability barrier from focal segmental glomerulosclerosis serum

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Background. Eicosanoids are believed to play a role in the pathophysiology of several models of glomerular disease. The cyclooxygenase inhibitor indomethacin reduces proteinuria in patients with focal segmental glomerulosclerosis (FSGS) or other glomerular diseases. We have shown that sera of some patients with FSGS significantly increase glomerular albumin permeability (P_{alb}) in an in vitro assay.

Methods. To determine the role of eicosanoids in the increased P_{alb} caused by the FSGS factor, glomeruli were isolated from normal rats, preincubated with indomethacin, then incubated with FSGS serum or normal serum and P_{alb} was calculated. To study the direct effect of individual eicosanoids on P_{alb} , glomeruli were incubated with prostaglandin E_2 , prostaglandin $F_{2\alpha}$ or a thromboxane A_2 mimetic, and P_{alb} was calculated. In the final set of experiments, normal glomeruli were preincubated with the thromboxane synthase inhibitor furegrelate, incubated with FSGS serum, and P_{alb} was calculated.

Results. Preincubation of isolated glomeruli with either the cyclooxygenase inhibitor indomethacin or the thromboxane synthase inhibitor furegrelate protected glomeruli from the increase in P_{alb} caused by FSGS serum. Each of the three principal glomerular eicosanoids significantly increased P_{alb} of isolated glomeruli.

Conclusions. These studies implicate a product of the cyclooxygenase pathway of arachidonic acid metabolism as mediating the increased P_{alb} caused by FSGS serum in our in vitro assay and possibly the proteinuria seen in patients with FSGS.

Primary focal segmental glomerulosclerosis (FSGS) is a glomerular disease characterized by heavy proteinuria, hypertension, and frequent progression to renal failure. It is a major cause of nephrotic syndrome in both children and adults, and its impact appears to be growing [1, 2]. Several investigators have reported an up to eightfold increase in the incidence of FSGS over the past 20 years

Key words: albumin, glomerular permeability, eicosanoids, cyclooxygenase inhibitor, proteinuria, arachidonic acid.

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[3–5]. The reasons for this observed increase are unclear at this time.

The etiology and pathogenesis of FSGS are unknown. A role for an etiologic factor in the circulation of some patients with FSGS has been proposed [6–11]. FSGS recurs in approximately 30% of patients who undergo renal transplantation, and in those patients who have had a prior recurrence FSGS returns in 80% of subsequent allografts. We have shown that sera from some patients with primary or recurrent FSGS increase the albumin permeability (P_{alb}) of isolated rat glomeruli in an in vitro assay [6].

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin inhibit cyclooxygenase in the arachidonic acid metabolism pathway. NSAIDs have been used in the treatment of patients with nephrotic syndrome [12, 13]. A beneficial effect of NSAIDs in reducing proteinuria in patients with primary or recurrent FSGS has been documented [12–15]. The purpose of these studies was to determine whether the cyclooxygenase inhibitor indomethacin alters the effect of FSGS serum on P_{alb} of isolated glomeruli. We also sought to determine if one or more of the primary arachidonic acid metabolites produced by glomerular cells alter P_{alb} of isolated glomeruli.

METHODS

Experimental animals

Normal male Sprague-Dawley rats (180 to 250 g) maintained on Purina rat chow and water ad libitum were used in all experiments. In all cases kidneys were removed via abdominal incision after the animals were properly anesthetized using metofane (Pitman Moore Inc., NJ, USA).

Isolation of glomeruli

After removal of the kidney capsule, the outer 1 to 2 mm of renal cortex was excised. Renal cortical slices were cut into fine fragments and the fragments were passed through consecutive screens of 80, 120 and 200

mesh size [16]. Glomeruli were recovered from atop the 200 mesh screen. Isolation of glomeruli was carried out at room temperature in medium which contained, in mmol/L: sodium chloride, 115; potassium chloride, 5; sodium acetate, 10; dibasic sodium phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 1.2; calcium chloride, 1; glucose, 5.5; L-alanine, 6; sodium citrate, 1; sodium lactate, 4. Bovine serum albumin (BSA) 4 g/dL was included in the medium as an oncotic agent. The pH of the medium was adjusted to 7.4. The oncotic pressure was measured using a membrane colloid osmometer (Model 4100; Wescor Inc., Logan, UT, USA).

FSGS serum

Serum from a patient who experienced a rapid recurrence of FSGS in a renal allograft was used in most experiments. This patient's serum has shown a consistently high level of activity (that is, the capacity to increase P_{alb}) in our in vitro assay, and has been used extensively as a positive control. Serum from each of four additional patients with FSGS also was used in further studies. In all experiments 1:50 dilution of FSGS serum (positive control) or serum from normal volunteers (negative control) was used.

Chemicals

Indomethacin and sodium furegrelate were obtained from Biomol Research Laboratories (Plymouth Meeting, PA, USA). Prostaglandin E_2 , prostaglandin $F_{2\alpha}$, and the thromboxane A_2 mimetic U46619 were obtained from Cayman Chemical (Ann Arbor, MI, USA). All other chemicals including 35% BSA solution were obtained from Sigma Chemical (St. Louis, MO, USA).

Treatment of glomeruli

Effect of indomethacin. In the first set of experiments, isolated glomeruli were preincubated with indomethacin at various concentrations (0.002, 0.01, 0.02, 1.0, 2.0, or 5.0 $\mu\text{mol/L}$) for 30 minutes at 37°C. These concentrations of indomethacin were chosen, as they are consistent with those that have been shown by other investigators to inhibit cyclooxygenase-mediated physiologic responses in glomeruli or glomerular cells [17–20]. FSGS serum or normal serum was then added, and glomeruli incubated for an additional 10 minutes at 37°C. In some experiments glomeruli were washed three times after preincubation with indomethacin with fresh isolation medium that contained no indomethacin, and then incubated with FSGS or normal serum. P_{alb} was then determined using volumetric change after bathing medium was changed to one of lower oncotic pressure (see below). In order to demonstrate that any observed effect was not restricted to the serum of a single patient, the experiment was repeated using serum from each of four additional patients.

Effect of individual eicosanoids. Glomeruli were isolated from normal rats as described above, and then incubated with various concentrations of prostaglandin E_2 (0.1, 0.5, 1.0, 5.0, 50 or 100 nmol/L), prostaglandin $F_{2\alpha}$ (0.5, 1.0, 5.0, 10, 20, 50 or 100 nmol/L) or the thromboxane A_2 mimetic U46619 (1.0, 5.0, 10 or 100 $\mu\text{mol/L}$) for 10 minutes at 37°C. These concentrations were chosen as they are well within the ranges that have been shown by other investigators to effect changes in glomerular cells [20–25]. In each set of experiments glomeruli were also incubated with FSGS or normal human serum as the positive or negative control, respectively.

Effect of furegrelate. Glomeruli were preincubated with various concentrations of the thromboxane synthase inhibitor furegrelate (0.04, 0.08, 0.2 or 0.4 $\mu\text{mol/L}$) for 30 minutes at 37°C, washed with fresh medium, then incubated with either FSGS serum or normal serum for 10 minutes at 37°C prior to determination of P_{alb} . These concentrations of furegrelate were chosen as they are within the range shown by other investigators to inhibit physiologic effects caused by thromboxane A_2 [26–28].

Measurement of glomerular volume response (ΔV)

The volume response of glomerular capillaries to an oncotic gradient was measured in the following manner [16]. Glomeruli were incubated in isolation medium containing normal serum, FSGS serum or various concentrations of prostaglandin E_2 , prostaglandin $F_{2\alpha}$ or the thromboxane A_2 mimetic U46619 for 10 minutes at 37°C. In some experiments glomeruli were preincubated with various concentrations of indomethacin or furegrelate as noted above. Glomeruli were then affixed to a glass coverslip coated with poly-L-lysine (1 mg/mL) and observed using videomicroscopy before and two to three minutes after the medium containing 4 g/dL BSA was replaced by medium containing 1 g/dL BSA. This exchange of medium produced an oncotic gradient across the glomerular capillary wall and resulted in net fluid influx and an increase in glomerular volume. Glomerular volume was calculated from the average of four diameters of the video image and the increase in volume (ΔV) of each glomerulus in response to an oncotic gradient was expressed as: $\Delta V = (V_{\text{initial}} - V_{\text{final}})/V_{\text{initial}} \times 100\%$

Reflection coefficient of albumin (σ_{alb})

There is a direct relationship between the increase in glomerular volume (ΔV) and the oncotic gradient ($\Delta\Pi$) applied across the capillary wall [16]. This principle was used to calculate σ_{alb} , using the ratio of ΔV of experimental to ΔV of control glomeruli in response to identical oncotic gradients: $\sigma_{alb} = \Delta V_{\text{experimental}}/\Delta V_{\text{control}}$

Convective permeability to albumin

Convective albumin permeability (P_{alb}) was defined as $(1 - \sigma_{alb})$ to describe the movement of albumin conse-

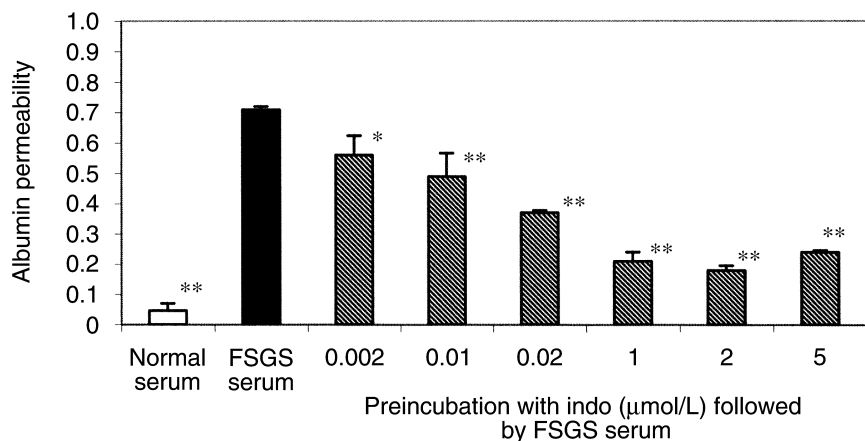


Fig. 1. Effect of indomethacin on increased albumin permeability (P_{alb}) caused by focal segmental glomerulosclerosis (FSGS) serum. Glomeruli were preincubated with indomethacin then incubated with FSGS serum and P_{alb} was determined. FSGS serum ($N = 37$) significantly increased P_{alb} compared to normal serum ($N = 32$). Indomethacin (0.002 $\mu\text{mol/L}$, $N = 7$; 0.01 $\mu\text{mol/L}$, $N = 6$; 0.02 $\mu\text{mol/L}$, $N = 14$; 1 $\mu\text{mol/L}$, $N = 14$; 2 $\mu\text{mol/L}$, $N = 6$; and 5 $\mu\text{mol/L}$, $N = 12$) protected the permeability barrier from the effect of FSGS serum (* $P < 0.01$ compared to FSGS serum, ** $P < 0.001$ compared to FSGS serum).

quent to water flow [16]. When σ_{alb} is zero, albumin moves at the same rate as water and P_{alb} is 1.0. Alternatively, when σ_{alb} is 1.0, albumin cannot cross the membrane with water and P_{alb} is zero.

Statistical analysis

Albumin permeability values are expressed as mean \pm SEM. N represents the number of glomeruli studied. Values among various groups were compared using ANOVA. Significance was defined as $P < 0.05$.

RESULTS

Indomethacin protects glomeruli from increase in P_{alb} caused by FSGS serum

Preincubation with indomethacin protected glomeruli from the increase in P_{alb} caused by FSGS serum. P_{alb} of glomeruli preincubated with indomethacin was significantly different from that of glomeruli incubated only with FSGS serum (positive control), and this difference was seen at all concentrations of indomethacin tested (Fig. 1). There was a trend toward greater protection at higher concentrations of indomethacin. In order to eliminate the possibility of a direct interaction between indomethacin and a substance in FSGS serum, glomeruli that had been preincubated with indomethacin (5 $\mu\text{mol/L}$) were washed with fresh medium that contained no indomethacin, then incubated with FSGS or normal serum. This treatment conferred a comparable degree of protection from the increase in P_{alb} caused by FSGS serum (FSGS serum 0.80 ± 0.04 , $N = 12$; unwashed glomeruli 0.15 ± 0.01 , $N = 12$; washed glomeruli 0.27 ± 0.06 , $N = 9$), with P_{alb} of both indomethacin treatments different from FSGS serum ($P < 0.01$).

To rule out the possibility that the glomerular response was specific to one patient's serum, isolated glomeruli were preincubated with indomethacin (1 $\mu\text{mol/L}$) and then incubated with serum from each of four additional

patients with FSGS. Indomethacin protected P_{alb} from the effect of FSGS serum (FSGS patient sera 0.77 ± 0.06 , $N = 23$ vs. preincubation with indomethacin -0.10 ± 0.08 , $N = 17$, $P < 0.01$).

Incubation with prostaglandin E_2 increases P_{alb}

Prostaglandin E_2 significantly increased P_{alb} of isolated glomeruli compared to normal serum (Fig. 2). This effect was seen at concentrations of prostaglandin E_2 as low as 0.5 nmol/L. The increase in P_{alb} caused by prostaglandin E_2 at concentrations of 5.0 nmol/L or greater was comparable to the increase caused by FSGS serum.

Incubation with prostaglandin $F_{2\alpha}$ increases P_{alb}

Prostaglandin $F_{2\alpha}$ significantly increased P_{alb} of isolated glomeruli compared to normal serum (Fig. 3). This effect was seen at concentrations of prostaglandin $F_{2\alpha}$ as low as 5 nmol/L. The increase in P_{alb} caused by prostaglandin $F_{2\alpha}$ at concentrations of 5.0 nmol/L or greater was comparable to the increase caused by FSGS serum.

Incubation with U46619 increases P_{alb}

The thromboxane A_2 mimetic U46619 significantly increased P_{alb} of isolated glomeruli compared to normal serum (Fig. 4). This effect was seen at concentrations of U46619 as low as 5.0 $\mu\text{mol/L}$. The increase in P_{alb} caused by the thromboxane A_2 mimetic at concentrations of 5.0 $\mu\text{mol/L}$ or greater was comparable to the increase caused by FSGS serum.

Furegrelate protects glomeruli from increase in P_{alb}

Preincubation with the thromboxane synthase inhibitor furegrelate protected isolated glomeruli from the increase in P_{alb} caused by FSGS serum (Fig. 5). P_{alb} was significantly different from that caused by FSGS serum at all concentrations tested. Furthermore, P_{alb} of glomeruli preincubated with furegrelate at a concentration of 0.4 $\mu\text{mol/L}$ was not different from negative control.

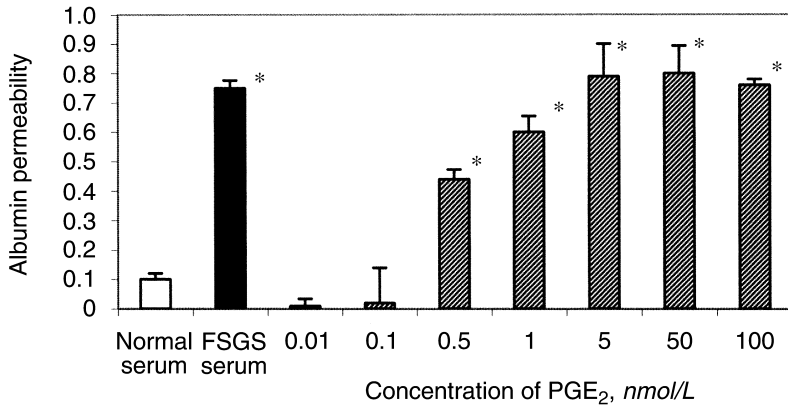


Fig. 2. Effect of prostaglandin E₂ (PGE₂) on P_{alb}. Glomeruli were incubated with various concentrations of prostaglandin E₂ and P_{alb} was determined. FSGS serum (N = 15) significantly increased P_{alb} compared to normal serum (N = 17). Prostaglandin E₂ increased P_{alb} compared to control at concentrations of 0.5 nmol/L (N = 12), 1 nmol/L (N = 8), 5 nmol/L (N = 9), 50 nmol/L (N = 9) or 100 nmol/L (N = 15). P_{alb} at concentrations of 0.01 nmol/L (N = 11) or 0.1 nmol/L (N = 11) was not different from control (*P < 0.001 compared to normal serum).

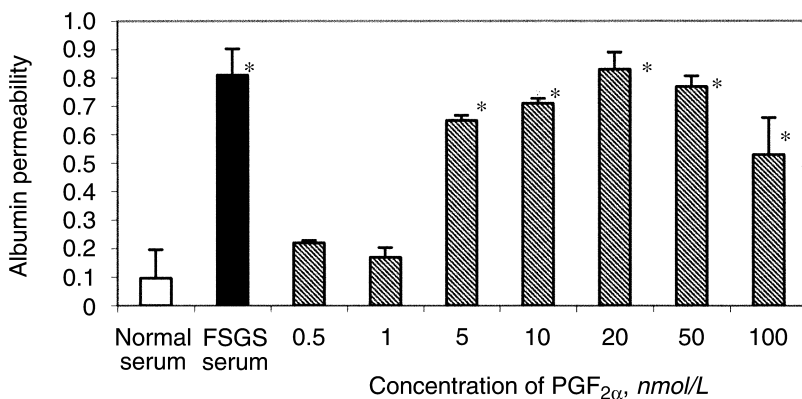


Fig. 3. Effect of prostaglandin F_{2α} (PGF_{2α}) on P_{alb}. Glomeruli were incubated with various concentrations of prostaglandin F_{2α} and P_{alb} was determined. FSGS serum (N = 9) increased P_{alb} compared to normal serum (N = 10). Prostaglandin F_{2α} increased P_{alb} compared to control at concentrations of 5 nmol/L (N = 11), 10 nmol/L (N = 11), 20 nmol/L (N = 12), 50 nmol/L (N = 17) or 100 nmol/L (N = 9). P_{alb} at concentrations of 0.5 nmol/L (N = 12) or 1 nmol/L (N = 11) was not different from control (*P < 0.001 compared to normal serum).

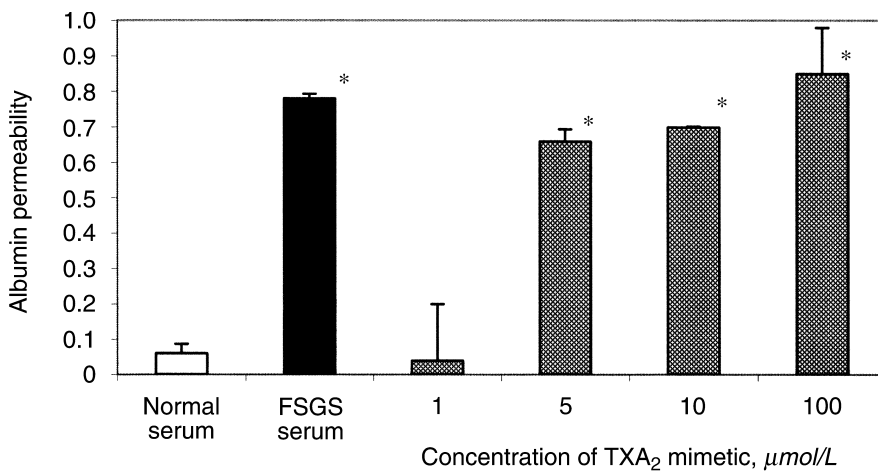


Fig. 4. Effect of the thromboxane A₂ (TXA₂) mimetic U46619 on P_{alb}. Glomeruli were incubated with various concentrations of U46619 and P_{alb} was determined. FSGS serum (N = 34) increased P_{alb} compared to normal serum (N = 34). The thromboxane A₂ mimetic U46619 increased P_{alb} at concentrations of 5 μmol/L (N = 33), 10 μmol/L (N = 9) or 100 μmol/L (N = 10) compared to control. P_{alb} at concentrations of 1 μmol/L (N = 10) was not different from control (*P < 0.001 compared to normal serum).

To rule out the possibility that the glomerular response was specific to one patient's serum, isolated glomeruli were preincubated with furegrelate (0.2 μmol/L) and then incubated with serum from each of four additional patients with FSGS. Furegrelate protected P_{alb} from the effect of FSGS serum (FSGS patient sera 0.77 ± 0.06, N = 23 vs. preincubation with furegrelate 0.11 ± 0.01, N = 20, P < 0.01).

DISCUSSION

These results show that the cyclooxygenase inhibitor indomethacin provides protection against the increased P_{alb} caused by FSGS serum in our in vitro assay. Additionally, we have demonstrated that incubation with any of the major glomerular eicosanoids, that is, prostaglandin E₂, prostaglandin F_{2α} or the thromboxane A₂ mimetic U46619, increases P_{alb} of isolated glomeruli. The throm-

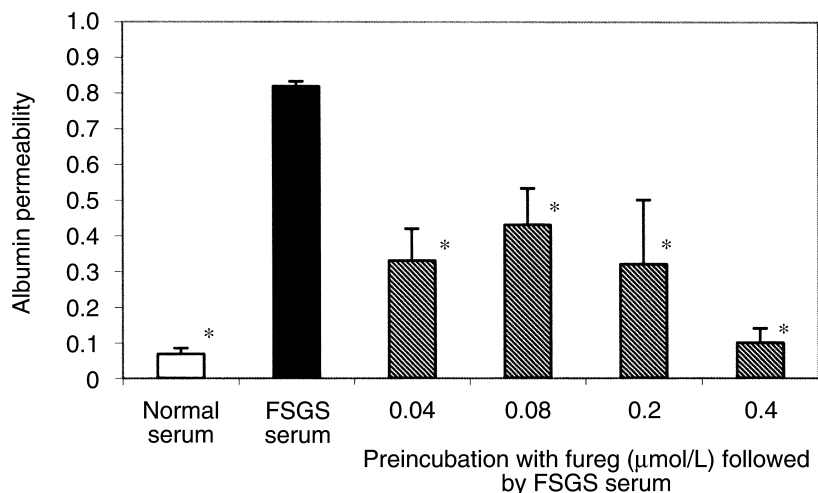


Fig. 5. Effect of furegrelate on increased P_{alb} caused by FSGS serum. Glomeruli were preincubated with the thromboxane synthase inhibitor furegrelate then incubated with FSGS serum and P_{alb} was determined. FSGS serum ($N = 20$) increased P_{alb} significantly compared to normal serum ($N = 19$). Furegrelate (0.04 $\mu\text{mol/L}$, $N = 5$; 0.08 $\mu\text{mol/L}$, $N = 5$; 0.2 $\mu\text{mol/L}$, $N = 5$ or 0.4 $\mu\text{mol/L}$, $N = 19$) protected the permeability barrier from FSGS serum (* $P < 0.001$ compared to FSGS serum).

boxane synthase inhibitor furegrelate also protects glomeruli from the increase in P_{alb} caused by the FSGS factor.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported for over 30 years to reduce proteinuria in patients with glomerular diseases [12, 13]. Investigators have described a reduction in proteinuria in nephrotic patients treated with NSAIDs, such as indomethacin (12–14, 29–31) or other NSAIDs [31–33]. This effect has been seen with both acute and chronic use of these drugs. As a rule, proteinuria reaches pretreatment levels with cessation of the NSAID.

NSAIDs have been used clinically to treat proteinuria seen in a variety of conditions. The beneficial effect of NSAIDs has been noted in primary [12, 13, 29–32] and recurrent FSGS [14, 15]. NSAIDs have been used to treat other glomerular diseases with a measure of success, including lupus nephritis [34], IgA nephropathy [35], membranous glomerulopathy [36, 37], and exercise-induced proteinuria [38].

Several investigators have examined the mechanism by which indomethacin and other NSAIDs reduce proteinuria. It has been generally held that cyclooxygenase inhibition alters relative and absolute levels of renal eicosanoids, which in turn leads to change in glomerular hemodynamics, specifically tone of the glomerular vasculature [12, 13]. Investigators have shown in animal models of renal disease that inhibition of eicosanoid production with the cyclooxygenase inhibitor indomethacin leads to a decrease in vasodilatory eicosanoids and subsequent increase in both afferent and efferent arteriolar tone [39–41]. It is proposed that in humans the reduction in glomerular filtration rate and intraglomerular pressure act to decrease proteinuria [42, 43].

In addition to its effects on hemodynamics, there is evidence that indomethacin alters the properties of the glomerular protein permeability barrier. Neugarten, Koz-

in and Cook showed that indomethacin significantly reduced the fractional clearance of neutral dextrans in a model of nephrotoxic serum nephritis [40]. The investigators theorized that indomethacin reduced the contribution of nonselective shunt pathways through which proteins leak out of glomerular capillaries. Golbetz et al treated nephrotic patients with indomethacin, and found a significant reduction in glomerular filtration rate (GFR) and renal plasma flow (RPF) after three days of treatment [44]. Additionally, indomethacin significantly decreased the fractional clearance of large dextrans. Al-lon, Pasque and Rodriguez concluded that addition of the NSAID ibuprofen enhanced the antiproteinuric effect of captopril not only by decreasing GFR, but also by enhancing permselectivity of the glomerular permeability barrier [45]. It has been noted by several investigators that though treatment with indomethacin decreased GFR, the proportional decrease in proteinuria was significantly greater than the decrease in GFR [37]. Moreover, the beneficial effect on proteinuria persists despite recovery in GFR [46, 47]. Together these studies support the notion that NSAIDs, indomethacin in particular, may alter the intrinsic properties of the glomerular protein permeability barrier.

Glomerular cells synthesize eicosanoids in health and disease. The major eicosanoids produced by glomerular epithelial, glomerular endothelial or mesangial cells are prostaglandin E_2 , prostaglandin $F_{2\alpha}$ and thromboxane A_2 [48–52]. Products of arachidonic acid metabolism, including those of the cyclooxygenase pathway, have been implicated in proteinuria seen in experimental glomerular disease. Remuzzi et al demonstrated that isolated glomeruli from rats made nephrotic after a single dose of the glomerular epithelial cell toxin adriamycin generated significantly more thromboxane B_2 (the stable metabolite of thromboxane A_2) than normal glomeruli [53]. Urinary excretion of thromboxane B_2 was increased

in nephrotic animals, and treatment of animals with a thromboxane synthase inhibitor decreased both proteinuria and urinary excretion of thromboxane B₂. Lianos, Andres and Dunn showed that isolated glomeruli from a model of nephrotoxic nephritis had a marked increase in thromboxane synthesis [54]. This increased synthesis was correlated with the degree of proteinuria. Alavi et al and Vriesendorp et al demonstrated a correlation between decrement in proteinuria and prostaglandin E₂ excretion in nephrotic patients treated with NSAIDs, including indomethacin [37, 55].

Indomethacin was shown to reduce proteinuria in passive Heymann nephritis without altering inulin clearance [56]. Cybulsky et al demonstrated that indomethacin decreased proteinuria and urinary prostaglandin E₂ excretion in a rat model of membranous glomerulopathy [57]. This group found that treatment with a thromboxane synthase inhibitor decreased proteinuria in these rats as well. Cybulsky et al then demonstrated that glomerular epithelial cells in culture release increased amounts of prostaglandin F_{2α} and thromboxane A₂ when incubated with anti-Fx1a antibodies and complement [58]. Eicosanoids are implicated as important mediators of proteinuria seen in other models of noninflammatory glomerular diseases, such as the remnant kidney model [59–63], proteinuria seen with high protein diet [64, 65] and exercise-induced proteinuria [66]. The central role played by eicosanoids, in particular thromboxane A₂, in anti-Thy-1 immune injury has been demonstrated by several groups [67–69].

We have demonstrated the presence of a substance in the plasma or serum (FSGS factor) of some patients with primary or recurrent FSGS that increases albumin permeability of isolated glomeruli in a unique bioassay. Presence of this FSGS factor is associated with and predictive of recurrence of disease in renal allografts [6]. The causative substance appears to be of low molecular weight, and we have succeeded in enriching this factor more than a hundred-fold [70]. The mechanism by which this FSGS factor alters the protein permeability barrier of the glomerulus is unknown, nor is it known which cell(s) of the glomerulus is affected by the FSGS factor.

In light of the existing body of evidence that eicosanoids alter glomerular physiology, we sought to determine if these substances are responsible for the increased glomerular permeability caused by the FSGS factor. Our results implicate a product of the cyclooxygenase pathway of arachidonic acid metabolism as a crucial mediator of the effect of the FSGS factor in serum to increase albumin permeability of isolated glomeruli. The unique *in vitro* bioassay eliminates any hemodynamic or humoral effect treatment with indomethacin may have when used in the intact animal or perfused kidney. Indomethacin appears to alter glomerular function in our studies by acting directly on cells of the glomerulus, as it

is unlikely that the structure of the glomerular basement membrane was substantially altered as a result of only brief *in vitro* exposure to indomethacin. Likewise, the hemodynamic or other systemic effects of furegrelate are obviated by using isolated glomeruli. Preincubation with indomethacin or furegrelate prevented the increase in P_{alb} caused by serum from each of four additional patients with recurrent FSGS, which demonstrates that the protection conferred by these drugs is not specific to a particular patient's serum.

We further demonstrate that each of the primary glomerular eicosanoids can increase glomerular albumin permeability and that this effect is once again independent of humoral or hemodynamic factors. The concentrations of the thromboxane A₂ mimetic U46619 that are required to cause increased P_{alb} are higher than those of either prostaglandin E₂ or prostaglandin F_{2α}, though are well within the range of concentrations that have been demonstrated to alter glomerular physiology [23–25]. Thromboxane A₂ is a highly labile compound compared with its stable mimetic U46619. U46619 differs chemically from native thromboxane A₂ in its ring structure. It is possible that this mimetic is relatively less active in our *in vitro* assay than the native compound. Alternatively, it is possible that thromboxane A₂ itself has less effect on the albumin permeability barrier than the other eicosanoids tested. However, the finding that inhibition of thromboxane synthase with furegrelate protects the permeability barrier from increased P_{alb} caused by the FSGS factor strengthens the conclusion that thromboxane A₂ may play an important role in the increased P_{alb}. It is possible that one or more of the major eicosanoids produced by glomerular cells are critical to mediate the increased P_{alb} seen *in vitro*.

We theorize that a factor present in the serum of some patients with FSGS may alter eicosanoid production of one or more glomerular cell types, leading to changes in the metabolism and function of cells of the filtration barrier. Inhibition of either cyclooxygenase or thromboxane synthase appears to prevent or possibly offset the change in eicosanoid metabolism caused by FSGS serum. Further studies are needed to understand the role played by various arachidonic acid metabolites in the increased P_{alb} seen *in vitro* and proteinuria in the clinical setting. Greater understanding of arachidonic acid metabolism in FSGS may lead to more effective treatment strategies.

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