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Transforming growth factor-β1 increases albumin permeability of isolated rat glomeruli via hydroxyl radicals

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Transforming growth factor-β1 increases albumin permeability of isolated rat glomeruli via hydroxyl radicals.

Background. Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine. Glomerular cells and tubular epithelial cells secrete and respond to TGF-β1. A close association between elevated levels of TGF-β1 and the development of glomerulonephritis, glomerulosclerosis, and tubular hypertrophy has been documented. The role of TGF-β1 in proteinuria is not well understood.

Methods. Isolated rat glomeruli were incubated in medium alone or with TGF-β1 (1 to 10 ng/mL) and TGF-β1 + 200 U/mL of superoxide dismutase (SOD) or 1 mmol/L of dimethylthiourea (DMTU) scavengers of superoxide and hydroxyl radicals, respectively, for up to 60 minutes at 37°C. Glomerular albumin permeability (P_{alb}) was calculated from the volumetric response of glomeruli to an oncotic gradient using videomicroscopy.

Results. One or 2.5 ng/mL of TGF-β1 had no effect on P_{alb} (0.18 ± 0.08, $N = 17$; 0.18 ± 0.079, $N = 20$ vs. control 0.00 ± 0.06, $N = 25$), whereas 5 or 10 ng/mL of TGF-β1 caused a significant increase in P_{alb} (0.31 ± 0.09, $N = 20$; 0.33 ± 0.06, $N = 23$) within 15 minutes. The effect of 10 ng/mL of TGF-β1 on P_{alb} increased further after 30, 45, or 60 minutes of incubation (0.43 ± 0.06, $N = 24$; 0.53 ± 0.06, $N = 25$; 0.74 ± 0.075, $N = 21$). The TGF-β1–induced increase in P_{alb} (0.75 ± 0.065, $N = 15$) was blocked by SOD (0.07 ± 0.14 $N = 15$) or by DMTU (0.04 ± 0.13, $N = 15$). Incubation of glomeruli with the carrier medium (4N HCl) in which TGF-β1 is dissolved and SOD or DMTU alone did not affect P_{alb}.

Conclusion. Elevated levels of TGF-β1 derived from glomerular or extraglomerular sources are capable of increasing glomerular P_{alb} via superoxide and hydroxyl radicals and may lead to proteinuria in vivo.

Transforming growth factor-β (TGF-β) is a multifunctional cytokine involved in the regulation of cell proliferation, differentiation, extracellular matrix (ECM) synthesis, and immune response [1, 2]. TGF-β is secreted by cells in a high molecular weight latent form, and its three components are (*1*) mature biologically active TGF-β, (*2*) latency-associated peptide (LAP), and (*3*) latent TGF-β–binding protein (LTBP) [3].

Transforming growth factor-β exists in three isoforms (TGF-β1, TGF-β2, and TGF-β3) with 65 to 85% homology. No discrete function for a specific isoforms has been identified [2]. Genes encoding TGF-β are located on three distinct chromosomes, are 100 kb (7 exons) in size with binding sites for transcription factors, and autoregulate their synthesis [4]. All three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) have been localized in kidneys, and their increased expression in human glomerulonephritis has been documented [5]. Increased expression of TGF isoforms and accumulation of ECM in acute and chronic renal transplant rejection have also been documented [6]. In numerous studies of experimental and human kidney diseases, TGF-β1 has been identified as a key mediator of glomerulosclerosis [7].

The biological effects of TGF-β1 are mediated by binding to specific cell membrane receptor proteins. Three types (I, II, and III) of TGF-β receptors have been identified [2, 8]. Types I and II are 53 and 75 kD glycoproteins, have serine threonine kinase activity in their cytoplasmic domains, and are present on all cells [9–12]. Type III receptor is a 280 to 330 kD proteoglycan with a short cytoplasmic domain [13]. Type I is involved in synthesis of matrix, type II in inhibition of growth, and type III in promoting the binding of TGF-β to receptors. Increased expression of all three types of TGF-β receptors in kidney sections from human glomerulonephritis patients has been documented [14].

Elevated levels of TGF-β1 have been associated with the glomerulonephritis [15], glomerulosclerosis or interstitial fibrosis [16], tubulogenesis [17], and tubular hypertrophy [18]. However, it is still unknown whether TGF-β1 plays a causative role in proteinuria. In the present study, we examined the direct effect of TGF-β1 on the glomerular filtration barrier and examined the role of reactive oxygen species (ROS) using in vitro measurements of glomerular albumin permeability (P_{alb}).

Key words: reactive oxygen species, glomerular albumin permeability, cytokine, proteinuria.

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METHODS

Experimental animals

Normal male Sprague-Dawley rats (180 to 250 g body weight) maintained on Purina chow and water ad libitum were used in all experiments.

TGF-β1 and reactive oxygen species scavengers

Human recombinant TGF-β1 was kindly supplied by Dr. Anita Roberts (National Institutes of Health, Bethesda, MD, USA). The biological activity of this TGF-β1 was measured in vitro by mink lung epithelial cell line assay, where a 50% inhibition was observed with 200 pmol/L (5 ng/mL) of TGF-β1. We have also tested the in vivo activity of this TGF-β1 [19]. The concentration used in most experiments was 10 ng/mL, which is within the physiologic range of the cytokine and is comparable to those used in other in vitro biological assays. ROS scavengers, superoxide dismutase (SOD) and dimethylthiourea (DMTU), and all other chemicals used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA). We used an excess amount of SOD (200 U/mL) and DMTU (1 mmol/L) to completely scavenge the superoxide or hydroxyl ion generated by TGF-β1.

Effect of TGF-β1 on glomerular albumin permeability

As described previously, glomeruli from Sprague-Dawley rats were isolated in medium containing 4 g/dL bovine serum albumin (BSA) [20]. In the first series of experiments, glomeruli were incubated with or without TGF-β1 (1 to 10 ng/mL) for 15 to 60 minutes at 37°C. In another set of experiments, glomeruli were incubated with TGF-β1 (10 ng/mL) alone or with 200 U SOD or with 1 mmol/L of DMTU for 45 minutes at 37°C. We also incubated some of the glomeruli with the carrier medium (4N HCl) in which TGF-β1 was dissolved as a control for 45 minutes at 37°C.

Measurement of glomerular volume change

After experimental treatment and incubation, glomeruli were allowed to adhere to coverslips coated with poly L-lysine (1 mg/mL) for 10 to 15 seconds. Images of adherent glomeruli were recorded using videomicroscopy. The initial medium was then replaced with fresh medium of lower oncotic pressure. Volume changes in glomeruli consequent to the applied oncotic gradient occurred within five seconds. Initial and final volumes of each glomerulus were calculated from the average diameter measured from the recorded images. Volume change (ΔV) was calculated as $\Delta V = (V_{final} - V_{initial}/V_{final}) \times 100$. At least five glomeruli from each of three to five rats were studied in each experimental condition.

Use of volume change to calculate σ_{alb}

The rationale and calculations for the measurement of σ_{alb} have been detailed in an earlier report [20]. Isolated nonperfused glomeruli exhibit a volumetric response to oncotic gradients. There is a direct relationship between the increase in glomerular volume (ΔV) and the oncotic gradient ($\Delta\pi$) applied across the capillary wall. In the current studies, $\sigma_{alb} = (\Delta V_{experimental}/\Delta V_{control})$. Convectional permeability (P_{alb}) is defined as $P_{alb} = (1 - \sigma_{alb})$.

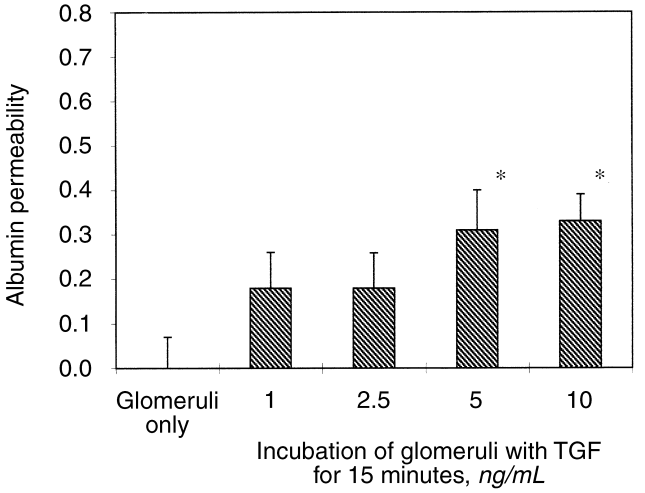

Fig. 1. Effect of various amounts of transforming growth factor-β1 (TGF-β1) on glomerular albumin permeability (P_{alb}). Rat glomeruli were incubated with or without 1 to 10 ng/mL of TGF-β1 for 15 minutes at 37°C, and P_{alb} was measured. As shown, 1 and 2.5 ng/mL of TGF-β1 had very little effect on P_{alb} ($N = 17$ and 20, respectively) compared with control glomeruli ($N = 25$). As shown, 5 and 10 ng/mL of TGF-β1 caused a significant increase ($^*P < 0.01$) in P_{alb} ($N = 20$ and 23, respectively).

Statistics

Average σ_{alb} and P_{alb} were calculated for glomeruli in each experimental condition, and values were compared using analysis of variance. Correlation coefficients were calculated from average values for each experimental condition. All values are expressed as mean ± SEM. P values < 0.05 were considered significant.

RESULTS

Effect of TGF-β1 on glomerular P_{alb}

Glomeruli were incubated with various concentrations of TGF-β1 (1, 2.5, 5, and 10 ng/mL) for 15 minutes at 37°C, and P_{alb} was calculated. As shown in Figure 1, TGF-β1 at concentrations of 1 or 2.5 ng/mL had no effect on P_{alb} (0.18 ± 0.08, $N = 17$; 0.18 ± 0.08, $N = 20$) compared with control (0.00 ± 0.07, $N = 25$). A significant increase in P_{alb} by TGF-β1 was evident at concentrations of 5 or 10 ng/mL (0.31 ± 0.09, $N = 20$, and 0.33 ± 0.06, $N = 23$, respectively) within 15 minutes. In other experiments, glomeruli were incubated with 10 ng/mL of TGF-β1 for 5, 15, 30, 45, and 60 minutes. As shown

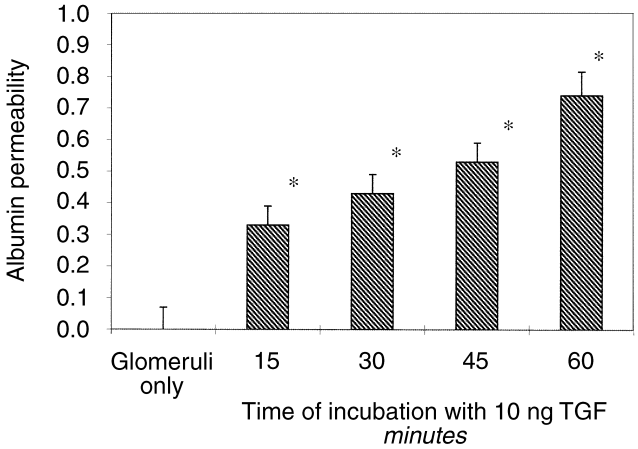

Fig. 2. Time course of TGF-β1–mediated increase in glomerular P_{alb}. Rat glomeruli were incubated with 10 ng/mL of TGF-β1 for up to 60 minutes, and the increase in P_{alb} was measured. TGF-β1 10 ng/mL caused a significant increase in P_{alb} (*$P < 0.01$) within 15 minutes ($N = 23$), and this effect of TGF-β1 increased further at 30 ($N = 24$), 45 ($N = 25$), and 60 minutes ($N = 21$) of incubation.

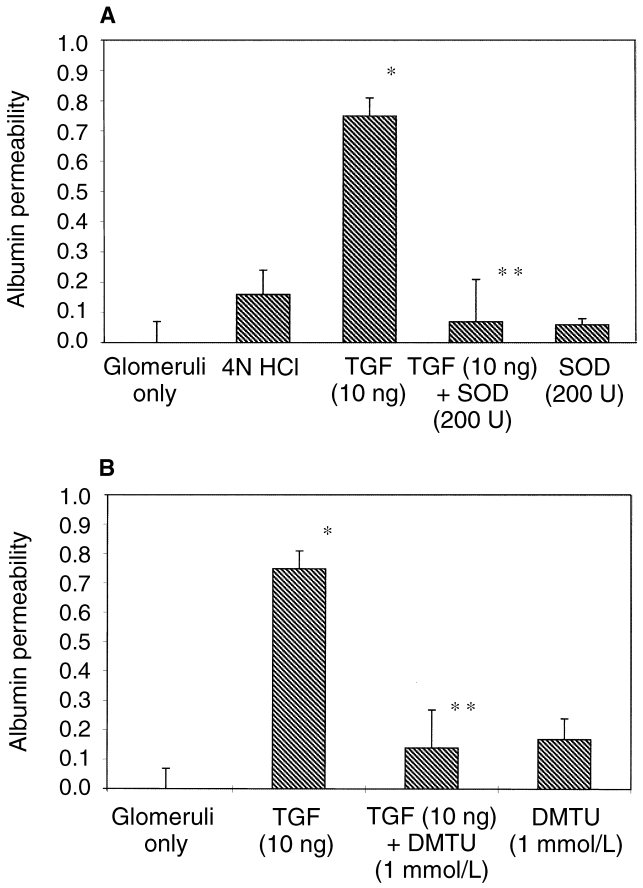

Fig. 3. Effect of reactive oxygen species (ROS) scavengers on TGF-β1 mediated increase in glomerular P_{alb}. Rat glomeruli were incubated in isolation medium, in control medium (4N HCl), with 10 ng/mL of TGF-β1 and TGF-β1 + SOD, or DMTU and SOD or DMTU alone for up to 60 minutes. (*A*) TGF-β1 caused a significant increase (*$P < 0.01$) in P_{alb} ($N = 15$), and SOD (200U/mL) blocked this effect ($N = 15$; shown by two asterisks. Four N HCl and SOD alone had no effect on P_{alb} ($N = 15$ and $N = 22$, respectively). (*B*) TGF-β1 caused a significant increase (*$P < 0.01$) in P_{alb} ($N = 15$), and 1 mmol/L of DMTU ($N = 15$) also blocked the TGF-β1–mediated increase in P_{alb}, as shown by two asterisks, and DMTU alone had no effect on P_{alb} ($N = 15$).

in Figure 2, 10 ng/mL of TGF-β1 caused a significant increase in P_{alb} within 15 minutes (0.33 ± 0.06, $N = 23$, vs. control 0.00 ± 0.070, $N = 25$), and this effect increased further in 30, 45, or 60 minutes of incubation at 37°C (0.43 ± 0.06, $N = 24$; 0.53 ± 0.06, $N = 25$; and 0.74 ± 0.075, $N = 21$, respectively).

Effect of oxygen radical scavengers on TGF-β1–mediated increase in P_{alb}

Further experiments were carried out to determine whether ROS were involved in the TGF-β1–mediated increase in P_{alb}. Glomeruli were incubated with TGF-β1 alone, TGF-β1 carrier medium (4N HCl), or TGF-β1 and ROS scavengers (SOD and DMTU). As shown in Figure 3A, TGF-β1 (10 ng/mL) induced an increase in P_{alb} with in 60 minutes (0.75 ± 0.06, $N = 15$, vs. control 0.00 ± 0.086, $N = 14$), and this effect was blocked by SOD (0.07 ± 0.14, $N = 15$). Incubations of glomeruli with TGF-β1 carrier medium, 4N HCl (0.16 ± 0.08, $N = 15$) and SOD alone had no effect (0.06 ± 0.02, $N = 22$) on $P_{alb.}$ As shown in Figure 3B, a TGF-β1–mediated increase in P_{alb} was also blocked by 1 mmol/L of DMTU (0.14 ± 0.13, $N = 15$), whereas DMTU alone had no effect (0.17 ± 0.07, $N = 15$) on P_{alb}.

DISCUSSION

Transforming growth factor-β1 significantly increased the P_{alb} of isolated rat glomeruli in this study. As little as 5 ng/mL of TGF-β1 increased P_{alb} within 15 minutes. The effect of TGF-β1 on P_{alb} was dose and time dependent. Incubation with 10 ng/mL of TGF-β1 for 45 minutes caused the maximal response in this study. SOD, a scavenger of superoxide, and DMTU, a scavenger of hydroxyl radical, abolished the TGF-β1–mediated increase in P_{alb}. These results implicate the hydroxyl radicals as important mediators in the effect of TGF-β1 on P_{alb}.

Proteinuria is a nonspecific manifestation of glomerular injury and is seen in systemic and renal diseases that are characterized by inflammation or elevated cytokine production such as platelet-activating factor (PAF) [21], tumor necrosis factor-α (TNF-α) [22], and TGF-β [23]. TGF-βs are homodimeric peptide growth factors with a molecular weight of 25 kD [2, 4]. TGF-βs are secreted by cells in a high molecular weight latent form, and their activation in vitro can be achieved by acidification, alkalization, and by proteases [3]. The latent form of

TGF-β is composed of three components: (*1*) mature biologically active TGF-β of 25 kD, (*2*) LAP, and (*3*) LTBP [3]. There are two kinds of TGF-β1, the large latent form with LTBP and small latent form without LTBP. Tubular cells secrete small latent TGF-β1, while glomerular cells secrete large latent TGF-β1 [24].

Elevated levels of circulating TGF-β1 have been implicated in the pathogenesis of tissue fibrosis in patients with advanced breast cancer [25] and thrombotic thrombocytopenic purpura [26]. Additionally, enhanced renal expression of TGF-β1 protein and mRNA has been reported in a range of glomerular diseases in both animal models [27–29] and in human disease [30, 31]. A close association between elevated expression of TGF-β1 and development of glomerulonephritis [15], glomerulosclerosis/interstitial fibrosis [16], tubulogenesis [17] and tubular cell hypertrophy [18] and tubular cell dysfunction [32] have been shown. Suppression of experimental glomerulonephritis by anti–TGF-β1 antiserum has also been shown [33]. In transgenic mice, increased levels of circulating TGF-β1 induced glomerular disease and proteinuria [34].

Transforming growth factor-β1 acts on glomerular cells in several ways. TGF-β1 stimulates production of ECM accumulation by glomerular epithelial cells [35], mesangial cells [36], and tubular epithelial cells [37]. The induction of ECM accumulation in these cells by TGF-β1 is achieved by three distinct mechanisms: (*1*) induction of transcription, synthesis, and secretion of matrix components, (*2*) decrease synthesis of protease's and increase in synthesis of protease inhibitors, and (*3*) increasing transcription and membrane expression of adhesion molecules (integrins) that regulate matrix assembly [2]. TGF-β1 stimulates mesangial cell proliferation through expression of platelet-derived growth factor protein and receptor [38]. TGF-β1 also inhibits production of inflammatory molecules such as interleukin-1 and inducible nitric oxide synthase [39]. TGF-β1 causes mesangial cell hypertrophy by inhibiting cell proliferation while increasing protein synthesis [40].

There is ample evidence that ROS are crucial mediators in inflammatory and noninflammatory glomerular disease [41]. Production of ROS is associated with increased P_{alb} in several animal models [42], and blocking the effects of these mediators with scavengers is associated with improvement of proteinuria [43]. Wang et al showed that treatment of puromycin aminonucleoside (PAN) nephrosis rats with cyclosporine A decreased proteinuria; treated rats also showed higher activities of glomerular SOD and catalase and attenuation of foot process effacement [44]. Ricardo, Bertram, and Ryan showed that the administration of SOD to rats with PAN nephrosis not only decreased proteinuria but also protected podocyte foot processes, as examined with electron microscopy [45]. We have shown that superoxide generated by either xanthine/xanthine oxidase system or by phorbol myristate acetate (PMA)-activated macrophages increases P_{alb} of isolated glomeruli, and this effect is abrogated by SOD but not catalase. These results indicate that superoxide is the mediator of proteinuria [46]. We have also shown that incubation of isolated glomeruli with PMA-activated rat polymorphonuclear cells increased P_{alb}, and this increase is prevented by catalase, SOD, taurine, or sodium azide, implicating hypohalous acid as the mediator of proteinuria [47].

Resident glomerular cells are capable of ROS production. Glomerular epithelial cells in culture produce ROS in response to various toxins such as doxorubicin and PAN [48, 49]. Mesangial cells in culture produce ROS in response to immune complexes [50], PMA, PAF [51], and TNF-α [52]. Augmented production of ROS by TGF-β1 in Hamester pancreatic beta-cell line HIT cells [53], in fetal hepatocytes [54], and in human lung fibroblasts [55] has been shown. The current results are consistent with the idea that TGF-β1 can also induce production of ROS by glomerular cells and thus can alter P_{alb}.

It is unlikely that new protein formation by glomerular cells or marked structural change of cells or basement membrane is responsible for the increase in P_{alb} induced by TGF-β1 in light of the short time of incubation. An increase in the message of collagen and fibronectin can be demonstrated only after four hours of incubation of glomerular epithelial cells with TGF-β1. We postulate that glomerular exposure to TGF-β1 stimulates the production of ROS, specifically superoxide and hydroxyl radicals, by mesangial cells or glomerular epithelial cells (GECs). ROS may then alter the properties of the GEC membrane, cytoskeleton, and/or intercellular junctions possibly by lipid peroxidation or may induce the production of other mediators such as eicosanoids, cyclic nucleotides, or cytokines, leading to increased P_{alb} in the experimental situation. Direct effects of TGF-β1 on the filtration barrier through a ROS mediator may explain proteinuria seen in settings of increased circulating TGF-β1 by infiltrating inflammatory cells and diseases with increased intrinsic glomerular TGF-β1 such as glomerulonephritis. We conclude that TGF-β1 is capable of affecting the glomerular filtration barrier directly without the involvement of secondary hemodynamic and immunologic effects, and that the TGF-β1–mediated increase in glomerular P_{alb} is independent of its effects on cell proliferation or on matrix synthesis.

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