View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 37 (1990), pp. 1048–1054

LABORATORY INVESTIGATION

Control of rat glomerular epithelial cell growth in vitro

STEPHEN ADLER, X. CHEN, and B. ENG

Division of Nephrology, Department of Medicine, New York Medical College, Valhalla, New York, USA

Control of rat glomerular epithelial cell growth in vitro. The interaction of cultured rat GEC[1] with several growth factors was explored in order to obtain a better understanding of in vivo factors which might stimulate GEC proliferation. GEC proliferated in response to EGF but not IGF-1, MSA or PDGF. Specific, saturable receptors for EGF were detected in saturation and competition binding studies utilizing ^{125}I-EGF with an approximate Kd of 1.7 nM and 86,000 binding sites per cell. TGF-β inhibited GEC growth in a time and dose dependent manner with a brief early exposure resulting in prolonged growth inhibition which was not reversible by EGF. Exposure to TGF-β sufficient to maximally inhibit growth had no effect on EGF binding to GEC. More prolonged exposure to TGF-β, however, did result in an increase in the apparent number of EGF receptors on GEC but no change in Kd. These studies suggest that EGF and TGF-β released by inflammatory cells or platelets during the course of glomerular injury may play a role in modulating glomerular cell proliferation.

Proliferation of glomerular cells occurs in response to renal injury in several different types of human and experimental renal disease. It is also an important component of the structural and functional alterations which lead to progressive loss of renal function even in the absence of acute ongoing injury [1]. Inflammatory cells, platelets and resident glomerular cells themselves are a potential source of growth factors and cytokines which may be important modulators of glomerular cell activity. Recent work utilizing cultured glomerular mesangial and epithelial cells has led to a better understanding of which of these factors may be involved in the initiation of glomerular cell growth in vivo [2–4].

Glomerular mesangial cells both secrete and proliferate in response to several cytokines and growth factors. Production of PDGF, IGF-1, IL-1 and GM-CSF by mesangial cells has been demonstrated by several groups [5–8]. Proliferation of mesangial cells is stimulated by several growth factors (EGF, PDGF, bFGF, IGF-1) and cytokines (IL-1, TNF) [5, 6, 9–12]. Several of these factors are released by macrophages and may explain some or all of the previously observed stimulatory effects of macrophage conditioned media on mesangial cell growth [2, 13]. Mesangial cell proliferation is inhibited by PGE_2, heparin and heparan sulfate in vitro and by heparin in vivo [14–17]. TGF-β displays a biphasic effect on murine mesangial cell growth in vitro while being inhibitory to epithelial and endothelial cells [18].

The factors which modulate glomerular epithelial cell growth have not been as extensively defined. Glomerular visceral epithelial cells (GEC) have been shown to proliferate in response to macrophage conditioned media and leukotrienes LTC_4 and LTD_4 [19]. The temporal association between influx of macrophages and crescent development in experimental models of crescentic nephritis also suggests that products of inflammatory cells may contribute to GEC proliferation, although the contribution of visceral epithelial cells, especially to inflammatory crescents, is not universally accepted [20]. We have previously shown that heparin and heparan sulfate are inhibitors of GEC growth and that EGF can partially reverse this inhibitory effect [21, 22]. To further explore factors which modulate GEC growth we have examined the response of GEC to several growth factors, have studied the binding of EGF to GEC and examined the growth inhibitory effects of TGF-β on these cells.

Methods

Reagents

Media and additives for culture of rat glomerular visceral epithelial cells were obtained from Gibco Laboratories (Grand Island, New York, USA), and Sigma Chemical Co. (St. Louis, Missouri, USA). Tissue culture plates and dishes were obtained from Becton Dickinson and Co. (Oxnard, California, USA). Epidermal growth factor (receptor grade-from mouse submaxillary glands; EGF), platelet-derived growth factor (from human platelets; PDGF), multiplication stimulating activity (from cultured Buffalo rat liver cells; MSA), insulin-like growth factor 1 (human recombinant; IGF-1) and transforming growth factor-β (from human platelets; TGF-β) were all obtained from Collaborative Research Inc. (Bedford, Massachusetts, USA). [^{125}I]-EGF was obtained from New England Nuclear (Boston, Massachusetts, USA).

Epithelial cell culture

Rat glomerular visceral epithelial cell (GEC) cultures were established from the kidneys of male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts, USA), cloned and characterized as previously described [3, 21]. Cells were maintained and passaged on collagen gels in a 50:50 mixture of K1 media (a defined epithelial cell growth mixture

[1] Abbreviations used are: bFGF-basic fibroblast growth factor; EGF-epidermal growth factor; GEC-glomerular visceral epithelial cells; GM-CSF-granulocyte macrophage colony stimulating factor; IGF-1-insulin-like growth factor-1; IL-1-interleukin-1; MSA-rat multiplication stimulating activity; PDGF-platelet-derived growth factor.

Received for publication May 10, 1989
and in revised form October 2, 1989
Accepted for publication October 10, 1989

© 1990 by the International Society of Nephrology

Table 1. Stimulation of GEC proliferation by growth factors

Factor	Relative increase in cell no. (mean ± SE)	N[a]
EGF *10 ng/ml*	2.7 ± 0.2	6[b]
EGF *100 ng/ml*	3.3 ± 0.2	6[b]
IGF-1 *100 ng/ml*	0.7 ± 0.2	6
MSA *100 ng/ml*	1.2 ± 0.1	6
PDGF *5 U/ml*	0.9 ± 0.1	6
PDGF *10 U/ml*	1.1 ± 0.2	5

GEC were plated in K1-0.5% FCS. Fresh media with the indicated growth factors was added after 4 to 6 hours. Cell number/well was determined by visual counting on days 1 and 3 post-plating and the increase in cell number with the factor compared to the increase in cell number in K1-0.5% FCS.

[a] Total number of wells studied

[b] $P < 0.05$ vs. control media

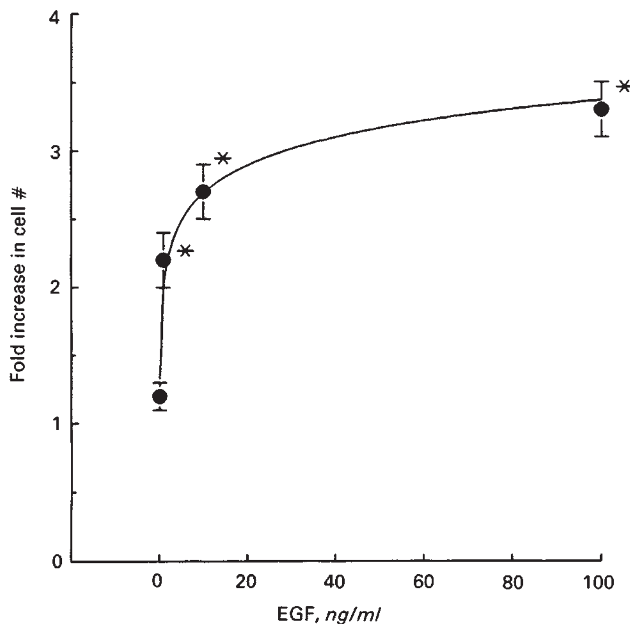

Fig. 1. *Proliferative response of GEC to EGF.* GEC were grown in K1-0.5% FCS with or without EGF at the indicated concentrations and the change in cell number/well from days 1 to 3 determined by visual counting. The ordinate represents the relative increase in cell number in EGF containing media compared to cells grown in K1-0.5% FCS. Each stimulus was evaluated in 3 wells/plate and the experiment was repeated twice. *$P < 0.02$ vs. control.

containing $NaHCO_3$-1.1 mg/ml) and conditioned media from Swiss mouse 3T3 fibroblasts (Dulbecco's MEM with 10% heat-inactivated fetal calf serum-FCS) [3]. Experiments were performed using cells plated on rat-tail collagen coated plates in their 15th to 25th passage.

Studies of GEC proliferation

Cells were plated at a density of 10 to 20,000/cm^2 on rat-tail collagen coated multi-well plates in K1 media containing 0.5% FCS. After the cells had adhered (4 to 6 hours) fresh media containing various concentrations of the growth factors under study was added. The growth factors and concentrations used are given in Table 1 and Figure 1. The number of cells/well was determined on day 1 and days 3 or 4 of growth by counting cells in a hemocytometer following their removal from wells with trypsin-EDTA. Stimulation of growth was assessed by comparing the increase in cell number in experimental versus control (K1-0.5% FCS) wells. Cells in experimental wells did not exceed 75 to 80% confluence when experiments were terminated. All stimuli were evaluated in three to four wells per plate and experiments were performed a minimum of two times. Because the concentration of insulin present in K1 media could potentially interact with receptors for insulin-like growth factors, experiments involving these factors were also repeated in K1 media lacking insulin.

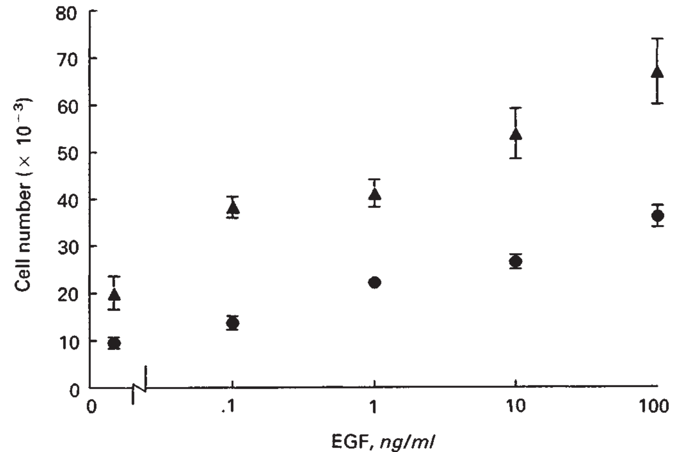

Fig. 2. *Effect of insulin on GEC proliferation.* GEC were grown in K1-0.5% FCS with (▲) or without (●) insulin and various concentrations of EGF as shown. Each point represents the mean ± SE of triplicate determinations of cell number/well on day 3. For points without error bars the SE was less than the symbol size.

Studies of growth inhibition

Cells were plated as above in K1-3T3 media. TGF-β at the indicated concentrations was added after four to six hours and change in cell number per well between days 1 and 3 determined as above. Inhibition of growth was calculated as follows: 1-[(change in cell number in K1-3T3 + TGF-β)/(change in cell number in K1-3T3)]. The effect of time of exposure to TGF-β on inhibition of GEC growth was also determined by exposing the cells to TGF-β for the times indicated below.

Measurement of EGF binding

Cells were plated at 10 to 20,000/cm^2 on rat-tail collagen coated plates in K1-3T3 media and allowed to grow for two days prior to study. In some experiments TGF-β (final concentration = 0.1 ng/ml) was added to cells four or 24 hours post-plating. Wells were washed twice with binding medium (Dulbecco's MEM + Ham's F12 (1:1) with 20 mM Hepes and 0.2% bovine serum albumin, pH 7.2) and then pre-incubated in binding medium for 45 minutes at 37°C with or without a 250- to 500-fold excess of unlabelled EGF. ^{125}I-EGF (100 to 200 μCi/μg; final concentration 4 ng/ml) was added to all wells and incubation continued for four hours at 0 to 4°C. Prior studies had demonstrated that binding was maximal by this time and that TCA precipitability of labeled EGF was constant during this period. Cells were then washed four times with PBS with 0.1% BSA,

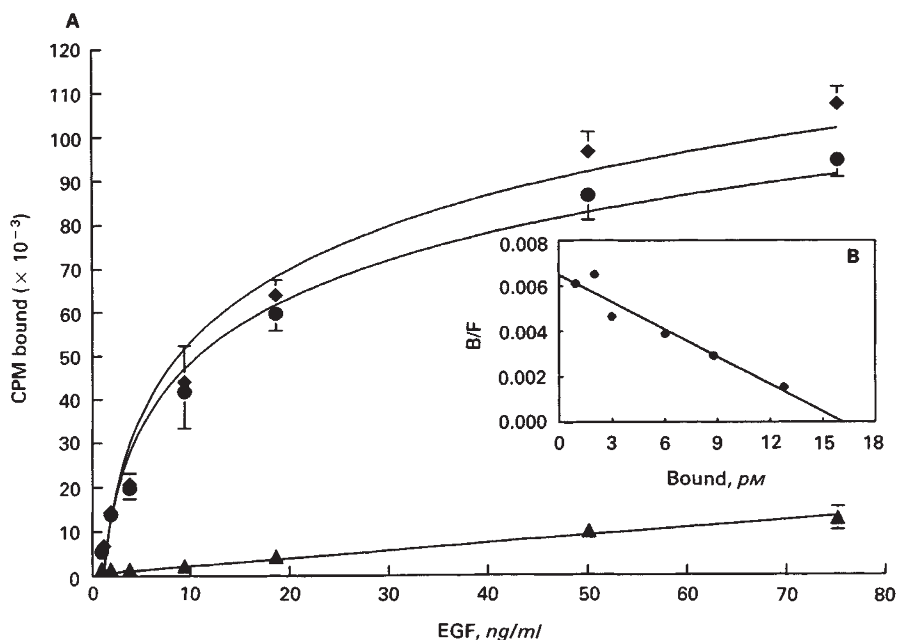

Fig. 3. *Binding of ^{125}I-EGF to GEC.* Symbols are: (●) specific; (◆) total, (▲) non-specific. **A.** GEC were incubated with increasing amounts of labelled EGF (1-75 ng/ml) for 4 hours at 0°C. Non-specific binding was determined in the presence of a 500-fold excess of unlabelled EGF. CPM bound are per 10^6 cells. Each point represents the mean ± SE of triplicate determinations performed twice. For points without error bars the SE was less than the symbol size. **B.** Scatchard transformation of the binding data.

solubilized in 1 N NaOH and transferred to tubes for counting. Non-specific binding to cells under these conditions was always <10%. Non-specific binding to the rat-tail collagen substrate was below the limits of detection. To determine whether changes in binding of ^{125}I-EGF reflected alterations in dissociation constant (Kd) or number of binding sites cells were incubated as above, but with varying amounts of ^{125}I-EGF up to 75 ng/ml. All results are expressed as specific binding (total − non-specific).

Analysis of results

All results are reported as mean ± SE. Comparisons of the means of groups were made using Student's *t*-test or one-way analysis of variance with post-hoc testing using the Tukey-Kramer test as appropriate, employing Systat statistical software (Systat Inc., Evanston, Illinois, USA). *P* values <0.5 were regarded as significant. Scatchard analysis of binding data was performed using the LIGAND program adapted for microcomputers (Elsevier Science Publishers BV, Amsterdam, Netherlands).

Results

Response of GEC to growth factors

The response of GEC to the growth factors studied is summarized in Table 1 and Figure 1. GEC proliferated in response to EGF with approximately a threefold maximum increase in proliferation compared to cells growing in K1-0.5% FCS (Fig. 1). A maximal response was seen with approximately 10 ng/ml EGF. No significant proliferative response was seen with MSA, IGF-1 or PDGF (Table 1). Because K1 media contains insulin (10 μg/ml) we also evaluated the response of GEC to EGF, MSA and IGF-1 in media without insulin. EGF was still able to promote GEC growth in the absence of insulin but an approximately twofold increase of cell number was seen in the presence of insulin at all levels of EGF studied (Fig. 2).

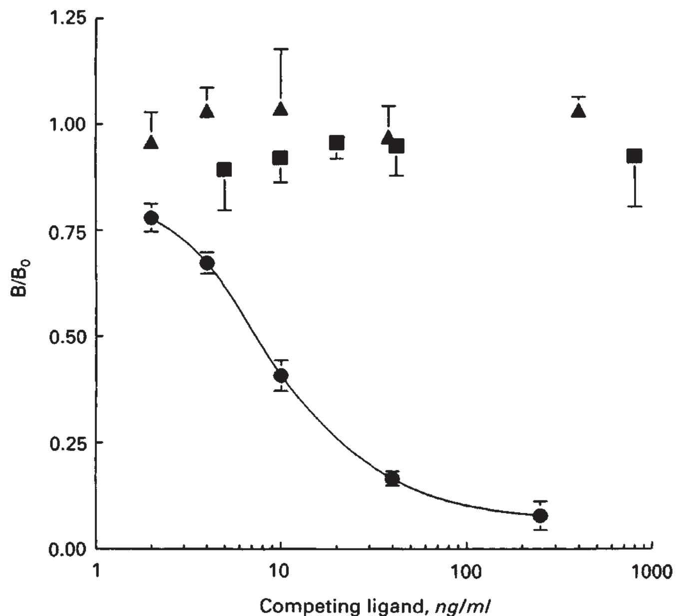

Fig. 4. *Competition for binding of ^{125}I-EGF to GEC.* Symbols are: (●) EGF; (▲) insulin; (■) TGF-β. GEC were incubated with ^{125}I-EGF (4 ng/ml) and increasing amounts of unlabelled competing ligand. Each point represents the mean ± SE of triplicate determinations performed twice.

MSA and IGF-1 still failed to stimulate GEC proliferation in this media (data not shown). Addition of IGF-1, MSA, PDGF, IGF-1 + PDGF, or MSA + PDGF to media containing EGF did not produce any further stimulation of growth above that obtained with EGF whether or not insulin was present in the media (data not shown).

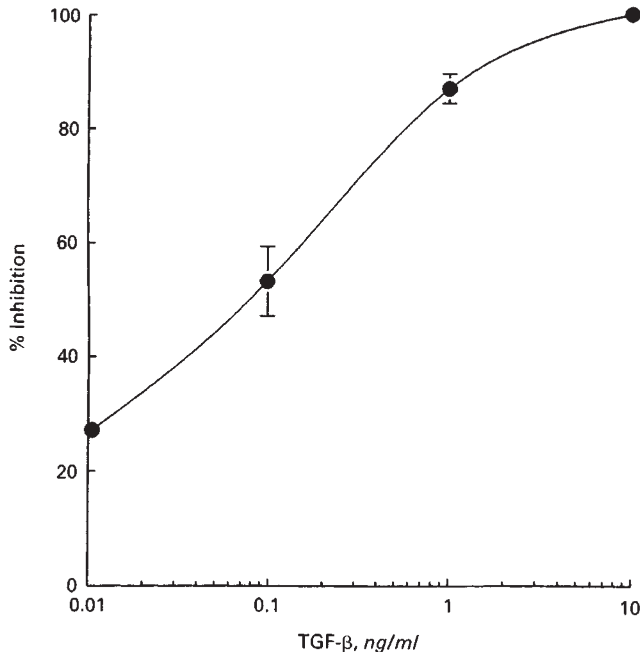

Fig. 5. *Effect of TGF-β on GEC growth.* Cells were plated in K1-3T3 media and TGF-β at the concentrations shown added after 4 to 6 hours. Inhibition of growth was determined from visual counts of cells on days 1 and 3. Each point represents the mean ± SE of quadruplicate determinations. The experiment was repeated twice with similar results.

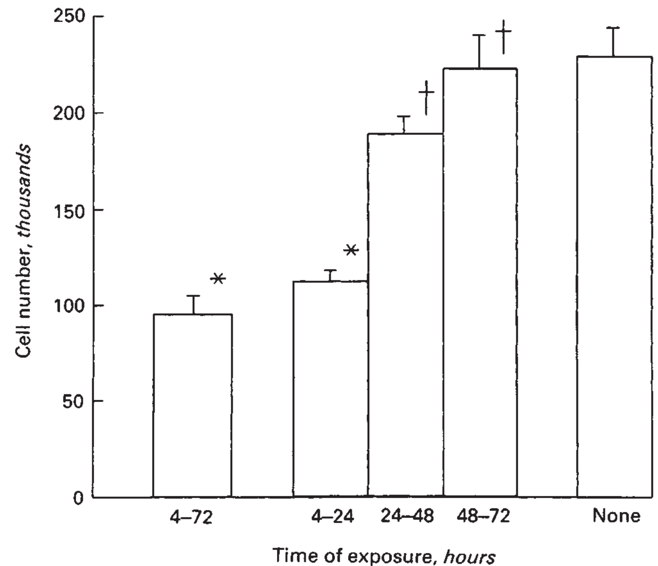

Fig. 6. *Effect of time of exposure to TGF-β on GEC growth.* GEC were plated in K1-3T3 media. TGF-β (0.1 ng/ml) was present in the media during the indicated time periods. Cell number/well was similar on day 1 for all the conditions studied. Cell number/well following 72 hours of growth was determined by visual counting. Each bar represents the mean ± SE of cells/well in 6 wells, and the experiment was repeated twice with similar results. (*$P < 0.001$ vs. no exposure to TGF-β; †$P > 0.2$ vs. no exposure to TGF-β).

EGF binding to GEC

^{125}I-EGF bound to GEC in a dose-dependent, saturable manner (Fig. 3A). Non-specific binding in the presence of a 500-fold excess of EGF was <10% except at the highest concentrations of ^{125}I-EGF used. Approximately 50% of the bound growth factor could be displaced within two hours by a 250-fold excess of cold EGF. Scatchard analysis of the binding data revealed a best fit to a one-site model with a dissociation constant (Kd) of approximately 1.7 nM and an apparent number of binding sites of 86,000/cell (Fig. 3B). Binding could be competitively inhibited by unlabelled EGF but not by other peptide growth factors such as insulin and TGF-β (Fig. 4).

Effect of TGF-β on GEC growth

Addition of TGF-β to GEC in K1-3T3 four to six hours following plating resulted in a dose-dependent inhibition of growth measured on the third day (Fig. 5). Addition of EGF (10 ng/ml) at the same time as TGF-β was not able to overcome this growth inhibitory effect (data not shown). When GEC were grown in K1-0.5% FCS supplemented with EGF (1 ng/ml) a similar inhibition of growth by TGF-β was seen (38.0 ± 6.8% inhibition at 0.1 ng/ml TGF-β; >90% inhibition at 1.0 ng/ml TGF-β). The time course of the effect of TGF-β on GEC growth was analyzed by exposing cells for different time periods during the three day period of study (Fig. 6). Exposure of GEC to TGF-β from 4 to 24 hours or from 4 to 72 hours of culture resulted in a significant decrease in cell number/well on day 3 ($P < 0.001$). However, the decrease in cell number/well on day 3 was similar with both of these conditions ($P > 0.4$), suggesting that a relatively brief, early period of exposure is necessary to achieve maximal inhibition of cell growth. Exposure of cells to TGF-β from 24 to 48 or 48 to 72 hours of the culture period produced progressively less inhibition of growth. Neither of these exposure times resulted in a significant decrease in cell number/well on day 3 ($P > 0.2$).

Table 2. Efffect of TGF-β on binding of ^{125}I-EGF to GEC

Time of exposure	CPM bound	
	Control	TGF-β
4 to 48 Hours	16304 ± 1103	27358 ± 1419[a]
24 to 48 Hours	13130 ± 1132	13925 ± 1089

GEC were plated in K1-3T3 media and grown in the presence of TGF-β (0.1 ng/ml) for the indicated times. Specific binding of ^{125}I-EGF (4 ng/ml) was determined following 48 hours of growth. The values shown are mean ± SE of specific CPM bound per 10^6 cells in triplicate wells. The experiment was repeated twice with similar results.

[a] $P < 0.05$ vs. control

Effect of TGF-β on binding of EGF to GEC

In view of the significant growth inhibitory effect of TGF-β on GEC and the importance of EGF in promoting growth of these cells we examined whether TGF-β might be acting by altering binding of EGF to its receptor on GEC. Cells growing in K1-3T3 media were exposed to TGF-β (0.1 ng/ml) beginning either 4 or 24 hours following plating and binding of EGF (4 ng/ml) was assessed after 48 hours of growth. Addition of TGF-β 24 hours after plating did not result in any significant change in the amount of EGF bound (Table 2). However, addition of TGF-β four hours after plating and continued exposure for the remaining 48 hours of growth did lead to a significant increase in binding ($P < 0.05$).

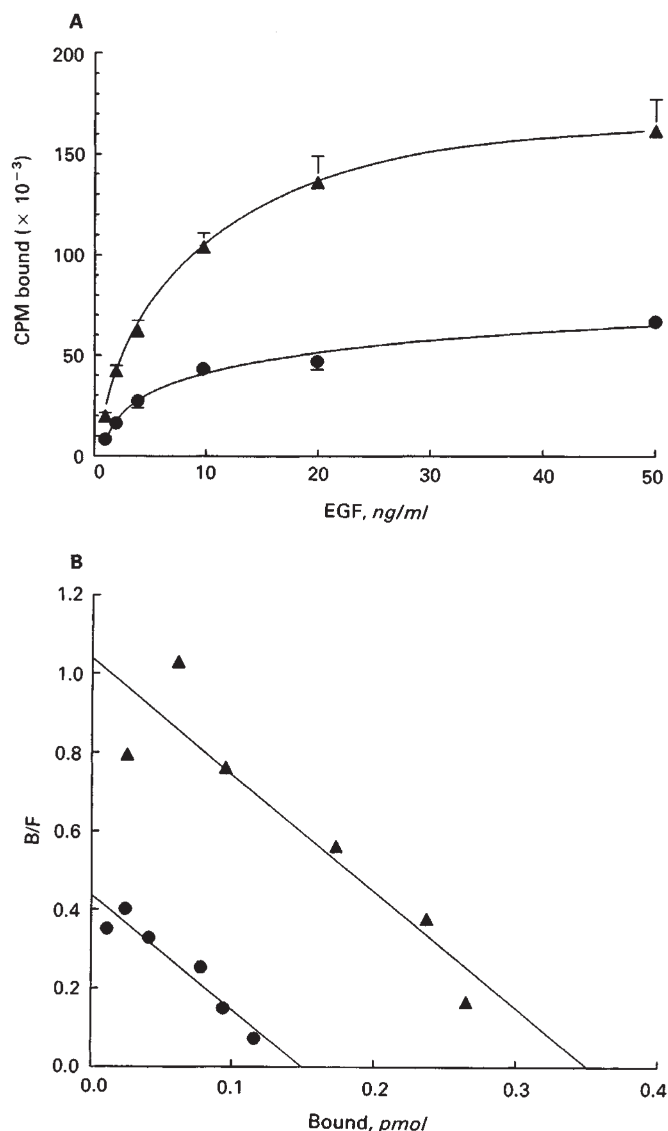

Fig. 7. *Effect of TGF-β exposure on binding of EGF to GEC.* **A.** GEC were grown for two days in K1-3T3 media in the presence of (▲) or absence (●) of TGF-β 0.1 ng/ml. Each point represents the mean ± SE of ^{125}I-EGF CPM bound per 10^6 cells in triplicate wells in two separate experiments. EGF binding was significantly greater in TGF-β treated cells at each level of EGF assayed ($P < 0.01$). **B.** Scatchard analysis of the binding data represented in A.

To determine whether this increase in binding of EGF represented an increase in number of cell surface EGF receptors or a change in the affinity of the receptor for EGF we performed saturation binding studies. GEC exposed to TGF-β (0.1 ng/ml) for 48 hours of culture were incubated with increasing amounts of ^{125}I-EGF (1 to 50 ng/ml) and specific binding determined. EGF binding was significantly increased by TGF-β exposure at each level of EGF added ($P < 0.01$; Fig. 7A). Scatchard analysis of the data (Fig. 7B) revealed similar Kd's (1.65 nM control; 1.40 nM TGF-β) but a rise in the mean number of apparent binding sites per cell (85878 control; 203242 TGF-β). Separate studies measuring binding of EGF to GEC at different cell densities per well showed a slight decrease in EGF binding with lower densities, suggesting that fewer cells per well in cultures exposed to TGF-β could not account for the observed rise in EGF binding (data not shown).

Discussion

The results presented here demonstrate that EGF is an important stimulus to GEC proliferation but that several other growth factors studied, including IGF-1, MSA and PDGF, failed to stimulate GEC growth. This is in contrast to glomerular mesangial cells which proliferate in response to several growth factors and cytokines. Saturation binding studies of ^{125}I-EGF to GEC reveal the presence of a single subclass of saturable binding sites with high affinity for EGF. This binding can be displaced by cold EGF but not by other polypeptide growth factors such as insulin or TGF-β. EGF receptors have also been demonstrated on intact rabbit glomeruli [23] and on glomerular mesangial cells, where they mediate several responses to EGF including growth, contraction and stimulation of Na^+/H^+ exchange [12]. A role for EGF stimulated Na^+/H^+ exchange in mediating cell proliferation in our experiments, however, is unlikely as they were performed in HCO_3^- containing media.

EGF may potentially be released during the course of glomerular injury from several sources. Both platelets and macrophages can produce EGF or EGF-like molecules [13, 24]. The presence of platelet fragments and platelet related antigens has been well established in several types of human and experimental glomerular disease [25, 26]. The presence of mononuclear cells and macrophages in glomeruli during the course of glomerulonephritis has been well demonstrated and they are also known to be an important constituent of glomerular crescents [20, 27]. The temporal association between macrophage ingress into glomeruli and the appearance of crescents has been taken as suggestive evidence that macrophage products may stimulate crescent formation, although the contribution of visceral epithelial cells, especially to inflammatory crescents, is not universally accepted [20]. Fibrin deposition in the urinary space has been widely held to be a stimulus to crescent formation; the evidence for this is indirect [28, 29]. Direct measurement of the effect of fibrinogen/fibrin and its breakdown products on GEC has not been done but the only effect seen on proliferation of glomerular mesangial cells was toxicity of some of the fibrin degradation products [30]. Release of EGF from macrophages is one potential mechanism whereby GEC might be stimulated to proliferate in vivo. Finally, the kidney itself is an important site of prepro-EGF production, although the localization of most of this to the thick ascending limb of the loop of Henle and the distal convoluted tubule do not readily suggest a pathway for it to reach glomerular cells [31, 32].

TGF-β was found to have a potent inhibitory effect on GEC growth, as it has on several other cell types including murine GEC [18, 33]. This effect is long lasting and occurs after a relatively brief exposure, similar to that observed in rat hepatocytes [34]. We did not find stimulation of growth by TGF-β at higher cell densities (unpublished observations) as has been reported by others with murine mesangial and rat aortic smooth muscle cells [18, 35], but did confirm the finding that TGF-β inhibits EGF stimulated growth of GEC as noted in murine GEC [18]. The inhibitory effect of TGF-β on GEC growth appears to occur independently of short term alterations in the

EGF receptor. Up to 24 hours of exposure to TGF-β produces no alteration in binding of ^{125}I-EGF to GEC despite maximal inhibition of growth, and addition of EGF does not reverse the effect of TGF-β. This would suggest that the action of TGF-β occurs at a later stage in the sequence of events leading to growth factor-induced cell proliferation. However, more prolonged exposure to TGF-β (44 hours) does increase the apparent number of EGF binding sites per cell. Other studies of the effects of TGF-β on EGF binding to cells have found either no effect [34, 36], decreased binding [37], increased binding [38] or a biphasic effect [39]. The studies performed here do not allow us to distinguish between an effect of TGF-β on receptor synthesis versus receptor internalization and recycling as an explanation for the increased binding at 48 hours.

In previous studies we examined the effect of several growth factors on GEC whose growth had been inhibited by heparin and found that EGF was capable of partially reversing heparin's effect [22]. In contrast to the inhibitory action of TGF-β on GEC, heparin's effect is, as noted, partially reversible by EGF and is associated with an increase in receptor affinity for EGF without any apparent change in number of receptors per cell. This, and the observation of a slight decrease in EGF binding with lower cell densities (unpublished observations) suggests that the increase in EGF binding to GEC exposed to TGF-β for prolonged periods is not simply a consequence of inhibited growth but must represent a specific effect of TGF-β. The significance of this finding for EGF action, however, is not clear.

It is likely that TGF-β is released during the course of glomerular injury from the same sources as EGF. Platelets are a rich source of TGF-β [40] and it has also been shown to be secreted by activated macrophages [41]. Furthermore, its presence has been detected in bovine kidney although its site of synthesis in the kidney has not been determined [42]. Whether it plays any physiologic role in modulating glomerular cell proliferation in vivo during the course of glomerular injury is unknown.

Acknowledgments

Portions of this work were presented at the 21st Annual Meetings of the American Society of Nephrology and have been published in abstract form (*Kidney Int* 35:339, 1989). This work was supported by grants from the National Institutes of Health DK01778 and DK40642 and by a grant from the Westchester Artificial Kidney Center. The authors thank L. Pinsker for technical assistance.

Reprint requests to Dr. Stephen Adler, Renal Unit, Westchester County Medical Center, Valhalla, New York 10595, USA.

References

1. Klahr S, Schreiner G, Ichikawa I: The progression of renal disease. *N Engl J Med* 318:1657–1666, 1988
2. Striker GE, Striker LJ: Glomerular cell culture. *Lab Invest* 53:122–131, 1985
3. Harper PA, Robinson JM, Hoover RL, Wright TC, Karnovsky MJ: Improved methods for culturing rat glomerular cells. *Kidney Int* 26:875–880, 1984
4. Lovett DH, Sterzel RB: Cell culture approaches to the analysis of glomerular inflammation. *Kidney Int* 30:246–254, 1986
5. Lovett DH, Larsen A: Cell cycle-dependent interleukin 1 gene expression by cultured glomerular mesangial cells. *J Clin Invest* 82:115–122, 1988
6. Shultz PJ, DiCorleto PE, Silver BJ, Abboud HE: Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. *Am J Physiol* 255:F674–F684, 1988
7. Conti FG, Striker LJ, Elliot SJ, Andreani D, Striker GE: Synthesis and release of insulin like growth factor I by mesangial cells in culture. *Am J Physiol* 255:F1214–F1219, 1988
8. Budde K, Coleman DL, Sterzel RB: Rat mesangial cells (MCs) produce a cytokine indistinguishable from granulocyte-macrophage-colony-stimulating factor (GM-CSF). (abstract) *Kidney Int* 35:342, 1989
9. Abboud HE, Throckmorton D, Weinshell E, Jaffer F: Differential modulation of mesangial cell proliferation by peptide growth factors and cyclic adenosine monophosphate (cAMP). (abstract) *FASEB J* 2:A627, 1988
10. Nakazato Y, Kester M, Mene P, Sedor JR: Tumor necrosis factor (TNF) stimulates mesangial cell (MC) proliferation independent of polyphosphoinositide turnover or phosphatidic acid formation. (abstract) *Kidney Int* 35:179, 1989
11. Doi T, Striker LJ, Elliot SJ, Conti FG, Striker GE: Insulin-like growth factor-1 is a progression factor for human mesangial cells. *Am J Pathol* 134:395–404, 1989
12. Harris RC, Hoover RL, Jacobson HR, Badr KF: Evidence for glomerular actions of epidermal growth factor in the rat. *J Clin Invest* 82:1028–1039. 1988
13. Nathan CF: Secretory products of macrophages. *J Clin Invest* 79:319–326, 1987
14. Ooi YM, Weiss MA, Hsu A, Ooi BS: Mechanisms of suppression of mouse mesangial cell proliferation by macrophage supernatants. *J Immunol* 130:1790–1795, 1983
15. Castellot JJ, Hoover RL, Harper PA, Karnovsky MJ: Heparin and epithelial cell secreted heparin-like species inhibit mesangial cell proliferation. *Am J Pathol* 120:427–435, 1985
16. Coffey AK, Karnovsky MJ: Heparin inhibits mesangial cell proliferation in Habu venom induced glomerular injury. *Am J Pathol* 120:248–255, 1985
17. Groggel GC, Marinides G, Hovingh P, Linker A: Heparan sulfate (HS) inhibits rat mesangial cell (MC) proliferation in culture. (abstract) *Kidney Int* 35:187, 1989
18. MacKay K, Striker LJ, Stauffer JW, Doi T, Agodoa LY, Striker GE: Transforming growth factor-β. Murine glomerular receptors and responses of isolated glomerular cells. *J Clin Invest* 83:1160–1167, 1989
19. Baud L, Sraer J, Perez J, Nivez M-P, Ardaillou R: Leukotriene C4 binds to human glomerular epithelial cells and promotes their proliferation in vitro. *J Clin Invest* 76:374–377, 1985
20. Cattell V, Jamieson SW: The origin of glomerular crescents in experimental nephrotoxic serum nephritis in the rabbit. *Lab Invest* 39:584–590, 1978
21. Adler S: Inhibition of rat glomerular visceral epithelial cell growth by heparin. *Am J Physiol* 255:F781–F786, 1988
22. Adler S, Eng B: Reversal of inhibition of rat glomerular visceral epithelial cell growth by growth factors. *Am J Pathol* (in press)
23. Redha R, Lopez C, Breyer JA, Jacobson HR, Breyer MD: Mapping of ^{125}-I epidermal growth factor binding sites along the rabbit nephron. (abstract) *Kidney Int* 35:319, 1989
24. Oka Y, Orth KN: Human plasma epidermal growth factor/β-urogastrone is associated with blood platelets. *J Clin Invest* 72:249–259, 1983
25. Duffus P, Parbtani A, Frampton G, Cameron JS: Intraglomerular localization of platelet related antigens, platelet factor 4 and β-thromboglobulin in glomerulonephritis. *Clin Nephrol* 17:288–297, 1982
26. Miller K, Dresner IG, Michael AF: Localization of platelet antigens in human kidney disease. *Kidney Int* 18:472–479, 1980
27. Schreiner GF, Cotran RS, Unanue ER: Macrophages and cellular immunity in experimental glomerulonephritis. *Springer Semin Immunopathol* 5:251–267, 1982
28. Naish P, Penn GB, Evans DJ, Peters DK: The effect of defibrination on nephrotoxic serum nephritis in rabbits. *Clin Sci* 42:643–646, 1972
29. Thomson NM, Simpson IJ, Peters DK: A quantitative evaluation of anticoagulants in experimental nephrotoxic nephritis. *Clin Exp Immunol* 19:301–308, 1975
30. Tsumagari T, Tanaka K: Effects of fibrinogen degradation prod-

ucts on glomerular mesangial cells in culture. *Kidney Int* 26: 712–718, 1984

31. Rall LB, Scott J, Bell GI, Crawford RJ, Penschow JD, Niall HD, Coghlan JP: Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. *Nature* 313:228–231, 1985
32. Salido ED, Yen PH, Shapiro LJ, Fisher DA, Barajas L: In situ hybridization of epidermal growth factor mRNA in the mouse kidney. (abstract) *Kidney Int* 35:319, 1989
33. Moses HL, Coffey RJ, Leof EB, Lyons RM, Keski-Oja J: Transforming growth factor β regulation of cell proliferation. *J Cell Physiol* (Suppl) 5:1–7, 1987
34. Russell WE: Transforming growth factor beta (TGF-β) inhibits hepatocyte DNA synthesis independently of EGF binding and EGF receptor autophosphorylation. *J Cell Physiol* 135:253–261, 1988
35. Goodman LV, Majack RA: Vascular smooth muscle cells express distinct transforming growth factor-β receptor phenotypes as a function of cell density in culture. *J Biol Chem* 264:5241–5244, 1989
36. Like B, Massague J: The antiproliferative effect of type β transforming growth factor occurs at a level distal from receptors for growth activating factors. *J Biol Chem* 261:13426–13429, 1986
37. Massague J: Transforming growth factor-β modulates the high-affinity receptors for epidermal growth factor and transforming growth factor-α. *J Cell Biol* 100:1508–1514, 1985
38. Shiota K, Nakamura T, Ichihara A: Distinct effects of transforming growth factor-β on EGF receptors and EGF induced DNA synthesis in primary cultured rat hepatocytes. *Biochem Int* 13: 893–901, 1986
39. Assoian RK: Biphasic effects of type β transforming growth factor on epidermal growth factor receptors in NRK fibroblasts. *J Biol Chem* 260:9613–9617, 1985
40. Assoian RD, Sporn MB: Type β transforming growth factor in human platelets: Release during platelet degranulation and action on vascular smooth muscle cells. *J Cell Biol* 102:1217–1223, 1986
41. Assoian RK, Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, Ross R, Sporn MB: Expression and secretion of type β transforming growth factor by activated human macrophages. *Proc Natl Acad Sci USA* 84:6020–6024, 1987
42. Roberts AB, Anzano MA, Meyers CA, Wideman J, Blacher R, Pan Y-CE, Stein S, Lehrman SR, Smith JM, Lamb LC, Sporn MB: Purification and properties of a type β transforming growth factor from bovine kidney. *Biochemistry* 22:5692–5698, 1983