Inhibition of heparin-binding epidermal growth factor-like growth factor increases albuminuria in puromycin aminonucleoside nephrosis

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Background. Our previous work in the acute puromycin aminonucleoside nephrosis (PAN) model has demonstrated up-regulation of heparin-binding epidermal growth factor-like growth factor (HB-EGF) mRNA and protein within glomerular epithelial cells (GECs) prior to the onset of proteinuria.

Methods. To determine whether increased HB-EGF expression in the acute PAN model contributes to the pathogenesis of proteinuria, a monoclonal antibody (DE10) was produced against recombinant human HB-EGF.

Results. The specificity of DE10 for human HB-EGF was confirmed by enzyme-linked immunosorbent assay, immunohistochemical staining, and flow cytometry of transfected cells expressing human and rat HB-EGF, and inhibition of cell proliferation. DE10 also reacted with cells transfected with rat HB-EGF cDNA. Administration of 0.5 mg affinity-purified DE10 to normal rats did not cause significant albuminuria compared with controls. Five days after the induction of the acute PAN model, albuminuria was significantly greater in animals treated with 0.5 mg DE10 than a control mAb (162.6 ± 32.4 vs. 64.8 ± 10.2 mg/day, respectively, P < 0.01). Rats treated with DE10 had an earlier onset of severe albuminuria, but no increase in maximal albuminuria at later time points. Electron microscopy showed marked podocyte effacement in both DE10-treated and control animals, but no obvious difference between groups. However, adhesion of the human GEC line 56/10 A1 to laminin and fibronectin, but not to collagens I or IV, was reduced by DE10.

Conclusions. This study suggests that HB-EGF contributes to the integrity of the glomerular filtration barrier, particularly when the podocyte has been injured. Following podocyte injury, adhesion to laminin in the glomerular basement membrane by HB-EGF may be important in reducing albuminuria.

Key words: HB-EGF, proteinuria, puromycin aminonucleoside, glomerular filtration barrier, VLA-3 adhesion molecule, F-actin microfilaments.

METHODS

Monoclonal antibody production

Female Balb/c mice (Monash University, Clayton, Victoria, Australia) were immunized with 10 μg recombi-
nant human HB-EGF (R&D Systems, Minneapolis, MN, USA) emulsified in complete Freund’s adjuvant (day 0) or incomplete Freund’s adjuvant (days 21 and 35) by intraperitoneal injection. Spleens were harvested three days after the third immunization and were fused with the nonsecreting mouse myeloma Sp2/0-Ag-14 using polyethylene glycol according to a published method [10]. Fusions were plated in 96-well plates at about 100 cells per well, and hybridomas were selected in hypoxanthine-aminopterin-thymidine (HAT). Wells containing hybridomas were screened by enzyme-linked immunosorbent assay (ELISA; discussed later in this article) against recombinant human HB-EGF. Positive wells were grown to 20 mL and frozen in liquid nitrogen. Cloning was performed by limiting dilution in 96-well plates using thymocyte feeders, as described by Oi and Herzenberg [11]. Antibody was used as spent hybridoma supernatants or purified ascitic fluid. Antibody subclass was determined by Ouchterlony immunodiffusion using a commercial mouse mAb typing kit (The Binding Site, Birmingham, UK).

Production and purification of ascitic fluids

Female Balb/c mice aged five months or more were used to produce ascitic fluids. 2,6,10,14-Tetramethylpentadecane (0.5 mL; Pristane; Sigma Chemical Co., St. Louis, MO, USA) was injected intraperitoneally on two occasions, seven days apart, followed by 1 to 5 × 10^6 hybridoma cells three days after the second injection. Mice were inspected daily for abdominal swelling and were sacrificed before there was evidence of distress. Ascitic fluids were collected by laparotomy, allowed to clot overnight, and further purified by ammonium sulfate precipitation. Thereafter, mouse IgG was purified by affinity chromatography on a HiTrap protein G column (Pharmacia Biotech, Uppsala, Sweden). The purified mAb was dialyzed against phosphate-buffered saline (PBS) prior to use. Purity of the affinity-purified mAb was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

ELISA for the detection of antibodies against EGF and related proteins

Enzyme-linked immunosorbent assay (ELISA) was performed as described by Engvall and Perlman [12]. Briefly, flat-bottomed PVC plates (Dynatech Laboratories Ltd., VA, USA) were coated with recombinant human HB-EGF, or the related proteins betacellulin (BTC), amphiregulin (AR), EGF, and transforming growth factor-α (TGF-α), by overnight incubation with the protein in carbonate buffer, pH 9.6. Plates were blocked with 1% bovine serum albumin (BSA)/PBS. Hybridoma supernatants were used undiluted. Antibody binding was detected using anti-mouse immunoglobulin coupled to horseradish peroxidase. Plates were developed using o-phenylenediamine hydrochloride (OPD), and the reaction was stopped with 4 N H_2SO_4. Plates were read on an automated plate reader (Behring EL 311 Microplate Reader; Behring, Behringwerke, Germany) at optical densities 490 and 630 nm.

Transfection of NIH-3T3, CHO, and Cos cells

To confirm mAb binding to rat and human HB-EGF, cells were transfected with one of two constructs. pHBE-EGF.hu was prepared by subcloning a 630 bp fragment of human HB-EGF cDNA (a generous gift from Dr. M. Gagnon in Professor M. Klagsbrun’s Laboratory, Children’s Hospital, Harvard Medical School, Boston, MA, USA) encoding the entire 208 amino acids, including the ATG start codon and the TGA stop codon. The fragment was subcloned into the expression vector pcDNA-3 (Invitrogen Corp., San Diego, CA, USA) to create the construct pH-EGF.hu. pH-EGF.rat was produced by first amplifying the coding sequence of rat HB-EGF using the primers 5’-ACCATGAGCTGGTCCGC-3’ and 5’-CATAGTCTGTTTGCACTTAGAGGC-3’ and subcloning this into pGEM-T (Pharmacia). The fragment was sequenced to confirm its identity, and the insert was then excised and subcloned into pcDNA-3 to create pH-EGF.rat.

For transfection, 2.2 × 10^6 cells/well were plated into six-well tissue culture plates in 2 mL of complete media in a CO_2 incubator at 37°C. Cells were incubated for 18 hours until 60% confluent. Two solutions were prepared: 2 mg of plasmid DNA were dissolved in 100 μL serum-free medium (Opti-MEM 1 reduced serum medium), and 12 μL of lipofectamine (Life Technologies, Gaithersburg, MD, USA) were dissolved in 100 μL serum-free medium. The two solutions were mixed gently and left at room temperature for 15 minutes; 0.8 mL of serum-free DMEM was then added to the mixed solution and overlaid onto washed cells for five hours at 37°C in the incubator. One milliliter of DMEM containing 20% fetal calf serum (FCS) was added, and the medium was replaced 24 hours later. For creation of stable transfectants, cells were selected in G418 and assayed when stable cell lines emerged.

Immunohistochemical staining of a transfected cell line

NIH-3T3 cells were transiently transfected with pHBE-EGF.hu or vector control using lipofectamine as described earlier. The cells were stained with the mAb DE10 using a three-step method. Briefly, the cells were grown on cover slips and fixed in acetone for five minutes at 4°C. DE10 as culture supernatant was added and incubated for one hour at 37°C followed by 30 minutes with a secondary antibody, rabbit anti-mouse Ig (Dako, Carpenteria, CA, USA) at 1:20 dilution and a further 30 minutes with APAAP complex (Dako) at 1:40 dilution. The slides were then developed in alkaline phosphatase.
substrate solution (Sigma) for 20 minutes and counterstained with hematoxylin and Scott’s tap water.

**Coupling of mAb to fluorescein isothiocyanate**

DE10 was purified with a protein-G column prior to conjugation. The antibody was dialyzed overnight against two to three changes of pH 9.5 carbonate/bicarbonate buffer (8.6 g NaCO₃ and 17.2 g NaHCO₃⋅1 L H₂O). The concentration of the protein was measured by optimal density at 280 nm, and 1 mg/mL of protein was used for conjugation. Fluorescein isothiocyanate (FITC) was dissolved in dimethyl sulfoxide (DMSO) to 10 mg/mL, and 100 µg were then added to the purified mAb dropwise, with stirring. The reaction was allowed to proceed for two hours shielded from light. After conjugation, excess dye was removed by gel filtration on Sephadex G-25 (PD10 columns from Pharmacia) in PBS containing 0.1% NaN₃. The absorbance of each conjugate was measured at 280 nm, and the conjugated antibody was stored at 4°C, protected from light.

**Flow cytometry**

Transfected cells were trypsinized and washed with PBS before incubating with FITC-labeled DE10, no antibody, and an irrelevant FITC-conjugated antibody for 30 minutes on ice. After washing the cells with PBS, the cells were resuspended in 400 µL of PBS and analyzed using a flow cytometer (FACSCalibur; Becton Dickinson, Sunnyvale, CA, USA).

**Cell proliferation assay**

Two × 10⁴ NIH-3T3 cells were placed in each well of 24-well tissue culture plates (Flow Laboratories, McLean, VA, USA) and allowed to adhere for 24 hours in 10% FCS in RPMI 1640. The cells were grown arrested for 48 hours in RPMI 1640 and then incubated with one of the following recombinant human growth factors; HB-EGF, EGF, BTC, AR, and TGF-α. Growth factors were all used at 10 ng/mL. ³H-thymidine 3 mCi (New England Nuclear, Boston, MA, USA) and the antibody test reagents at 1 mg/mL were also added at the same time. Cultures were incubated for 24 hours at 37°C in 5% CO₂. The cell layers were then washed, harvested with 200 µL NaOH, and harvested onto glass fiber filter paper using a cell harvester (Inotech Cell Harvester System; Inotech Biosystems International, Rockville, MD, USA). The filter paper from each well harvested was then mixed with 50 µL liquid scintillant, and specific activity was counted using a β counter (Packard Tri-Carb 1600CA).

**Cell adhesion assays**

Cell adhesion assays were performed as described by Chakravarti, Tam, and Chung using the human GEC line 56/10 A1 [13, 14], a generous gift of Professor J.D. Sraer. Twenty-four-well tissue culture plates were coated with the relevant protein through the addition of 100 µL of a 10 µg/mL solution and incubation for two hours at 37°C. The following extracellular matrix constituents were used; bovine collagen I (Vitrogen; Collagen Corporation, Palo Alto, CA, USA), mouse type IV collagen IV (Life Technologies), human placental laminin (Sigma), and bovine plasma fibronectin (ICN Biomedicals Inc., Aurora, OH, USA). Nonspecific adhesion was blocked by incubating the plates in 1% bovine serum albumin in PBS at 4°C overnight. Cell monolayers were radiolabeled with ³H-thymidine (2.5 mCi/mL) overnight and then briefly trypsinized. The cells were resuspended in RPMI media containing 1% fetal calf serum and incubated with the monoclonal antibodies DE10, OX-34 (Serotec Ltd., Kidlington, Oxford, UK), or anti-CD49c (the α chain of VLA-3) antibody (11G5; Serotec) at 4 µg/mL for approximately 15 minutes. The radiolabeled cells were then added to the wells at 1 × 10⁶ cells/well and then incubated at 37°C for one hour to allow the initial attachments to form. Unattached cells were removed by washing with PBS, and the attached cells were solubilized in 1 mol/L NaOH. Incorporation of the ³H-thymidine was determined using a cell harvester and liquid scintillation β-counter, as before. Results are expressed as the percentage of binding compared with the no-antibody control.

**The acute PAN model**

Acute PAN was induced in 150 to 170 g Sprague-Dawley rats using an established protocol [7] by a single intravenous injection of 15 mg/100 g PAN (Sigma). At least six rats were used per group.

**Administration of mAb to rats**

Male Sprague-Dawley rats (150 to 170 g) were injected intravenously with a single dose of 0.5 mg of DE10, the irrelevant mAb RMAC 8 (IgG2a), or saline. For rats with PAN, the antibody was administered the day following injection of PAN. Urine was collected for 16 hours in metabolic cages. Albuminuria was measured by radial immunodiffusion using commercial plates incorporating an anti-rat albumin antibody (rat/mouse albumin NL RID plates; The Binding Site).

**Detection of DE10 in the serum of normal rats**

Male Sprague-Dawley rats (N = 3) weighing approximately 150 g were injected intravenously with 0.5 mg DE10. Blood was collected at various times, and the injected DE10 was detected in rat serum using ELISA. Briefly, plates were coated with 10 µg/mL goat anti-mouse Ig, blocked, and then reacted with diluted (1:400) rat serum collected between days 0 and 35. Noninjected rats were also included as controls. Plates were then incubated with biotinylated swine anti-mouse Ig fol-
ollowed by Streptavidin peroxidase. Peroxidase labeling was revealed using OPD. The reaction was stopped using 4 mol/L sulfuric acid and absorbances read at 492 nm on an ELISA plate reader (Behring). Known concentrations of the injected antibody were used to generate a standard curve and the concentration of DE10 in rat serum calculated.

**Fluorescence microscopy**

Following induction of the PAN model of proteinuria, rats weighing approximately 150 g were injected intravenously with 1.5 mL saline, RMAC 8 0.5 mg, or DE10 0.5 mg, and then were sacrificed three days later. Kidneys were harvested and snap frozen in liquid nitrogen, and 4 μm cryostat sections were prepared and fixed in ice-cold acetone for 10 minutes at 4°C. The sections were blocked in 3% BSA/5% rat serum/PBS for 30 minutes at room temperature and then stained using anti–WT-1 rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a final concentration of 1 μg/mL. Sections were washed in PBS and then stained with Oregon Green-conjugated goat anti-mouse antibody at a final concentration of 5 μg/mL (Molecular Probes, Eugene, OR, USA) and Texas red–X conjugated goat anti-rabbit antibody at a final concentration of 2 μg/mL (Molecular Probes). Sections were comprehensively washed in PBS and then mounted with fluorescent mounting medium (Dako). Images were collected and generated on a confocal laser scanning microscope (Bio-Rad MRC 1024; Bio-Rad Microscopy Division, Hemel Hempstead, Herts, UK).

**Transmission electron microscopy**

Tissue was immersion fixed in 2.5% glutaraldehyde for two hours, postfixed in 1% osmium tetroxide, dehydrated in graded acetones, and embedded in Epon-Araldite. Sections (0.08 μ) were cut and stained with uranyl acetate and lead citrate and examined in a Phillips CM12 electron microscope.

**Statistics**

Statistics were performed using the Instat software package, version 2.01, from GraphPad Software. Multiple comparisons were performed using analysis of variance (ANOVA), followed by the Tukey–Cramer multiple comparison test. A P value <0.05 was considered significant.

**RESULTS**

**Production and characterization of mAb DE10**

A total of 1540 wells were screened and 77 positive wells obtained. The mAb DE10 was selected for cloning because it was the only antibody that could be shown to stain tissue sections by immunohistochemistry. It was found to be IgG2a. In ELISA, DE10 was strongly positive against rhHB-EGF but did not react with rhEGF, rhTGF-α, rhAR, or rhBTC (Fig. 1). Reactivity with HB-EGF was confirmed by immunohistochemical staining of NIH-3T3 cells transiently transfected with a construct containing human HB-EGF cDNA (pHB-EGF.hu) using the APAAP technique (Fig. 2). Cells transfected with a vector control were negative. Moreover, FITC-conjugated DE10 also reacted in flow cytometry with CHO cells stably transfected with pHB-EGF.hu (Fig. 3A). DE10 also bound Cos cells stably transfected with a construct encoding rat HB-EGF (pHB-EGF.rat) by flow cytometry (Fig. 3B). In both of these assays, DE10 did not bind cells transfected with a vector control.

**Functional activity of DE10**

To determine whether DE10 inhibited the functional activity of HB-EGF, DE10 or an irrelevant mAb of the same isotype was added to NIH-3T3 cells stimulated with HB-EGF, EGF, TGF-α, AR, or BTC. Cell proliferation was measured by incorporation of 3H-thymidine. There was significant inhibition of cell proliferation when the cells were treated with DE10 and stimulated with HB-EGF, but not with an irrelevant mAb (Fig. 4). Also, DE10 had no effect when cells were stimulated with other members of the EGF family. Interestingly, inhibition of proliferation with DE10 was not complete, despite an approximately tenfold molar excess.

**Treatment of rats with DE10**

The serum half-life of DE10 in normal rats, following administration of 0.5 mg affinity-purified DE10, was eight days (Fig. 5). When administered to normal rats, DE10 did not produce significant albuminuria. When
Fig. 2. Immunohistochemical staining of NIH-3T3 cells transiently transfected with a mammalian expression vector without an insert (vector control, pcDNA-3; A) or a vector containing a full-length cDNA for the coding region of human HB-EGF (pHB-EGF.hu; B). The red stain is positive (APAAP method, x100).

Fig. 3. FACS histograms of FITC-conjugated DE10 binding to (A) Chinese hamster ovary (CHO) cells stably transfected with pcDNA-3 (vector control, green line) or pHB-EGF.hu (red line), and (B) Cos cells stably transfected with pcDNA-3 (green line) or pHB-EGF.rat (red line).

Fig. 7. Localization of DE10 within the glomerulus of PAN-treated rats. Rats treated with PAN were injected with saline (A), RMAC 8 (B), or DE10 (C) and sacrificed three days later. Kidney sections were stained for the presence of administered antibody (anti-mouse Ig; green fluorescence) and for podocyte nuclei (WT1; red fluorescence). No green fluorescence is seen in rats injected with saline (A). Green fluorescence, predominantly within the lumina of capillaries, is seen in rats injected with RMAC 8 (B). Areas of colocalization of antibody and podocyte resulted in the accumulation of emission, which appeared as yellow and are indicated with arrows (C).
Fig. 4. Cell proliferation assay, measured using 3H-thymidine incorporation, of NIH-3T3 cells stimulated with growth factors of the EGF family or no growth factor (Neg). Cells were stimulated with 10 ng/mL of each growth factor and saline or mAbs DE10 (■) or RMAC 8 (□) added at 1 µg/mL. *P < 0.0001. The error bars are ±1 SD.

Fig. 6. Treatment of rats with 0.5 mg DE10. (A) Rats injected with DE10 the day after induction of the PAN model (that is, on day 1) and studied at five-day intervals thereafter. (B) A second group of rats studied daily over the first five days postinduction of the PAN model. *P < 0.01. Symbols are rats treated with: ■ RMAC 8; □ DE10; or ▲ receiving no treatment. The error bars are ±1 SD.

Fig. 5. Serum levels of DE10 in normal rats following injection of DE10. Rats were injected with 0.5 mg DE10 (N = 3), and the data were fit to the curve of best fit using Microsoft Excel. The error bars are ±1 SD.

administered the day following injection of PAN 15 mg/kg, there was an earlier onset of proteinuria (Fig. 6), commencing at day 3 compared with day 5 in the control rats (Fig. 6B), and a significantly greater level of proteinuria at day 5 (Fig. 6A). By day 10, this had fallen to levels comparable with control animals. When DE10 was administered at day 5, however, there was no difference in proteinuria five days later (data not shown). To determine whether the increase in proteinuria was due to complement-mediated injury to podocytes by DE10, animals were complement-depleted using cobra venom factor (CVF) administered on alternate days and then treated with DE10 after induction of the PAN model, as before. Complement depletion was checked prior to administration of each dose by CH50 and was found to be complete. There was no difference in the onset of proteinuria in animals receiving CVF, PAN, and DE10 compared with animals receiving only PAN and DE10 (data not shown).

Localization of DE10 in the kidneys of treated rats by laser scanning confocal microscopy

Rats injected with PAN were treated on the following day (day 1) with saline (Fig. 7A), the irrelevant mAb RMAC 8 (Fig. 7B), or DE10 (Fig. 7C), and sacrificed on day 4. Antibody detected in kidney sections were stained with anti-mouse Ig and the sections were costained with a rabbit polyclonal antibody directed against the podocyte marker WT1. Mouse Ig staining, in rats injected with RMAC 8, was localized predominantly to capillary loops
(Fig. 7B). The same staining was seen in rats injected with DE10, but in addition, there were cells that stained with both WT1 and anti-mouse Ig (Fig. 7C), demonstrating that DE10 localized specifically to podocytes.

**Electron microscopy of kidneys from rats treated with DE10**

To determine whether the severity of proteinuria at day 5 was reflected by a difference in morphology, groups of three animals with PAN, which received either RMAC 8 or DE10 at day 1 postinduction, were killed at days 3 and 5, and the kidneys were fixed for electron microscopy. The appearances of the kidney at day 3 were similar in both groups, although focal foot process effacement and mild swelling of podocytes were only apparent in the animals that received DE10; the animals that received RMAC 8 showed relatively normal glomerular morphology at this stage (Fig. 8 A, B). However, at day 5, both groups showed widespread severe effacement of foot processes, associated with extensive swelling, vacuolation and protein droplet accumulation within the cytoplasm of podocytes (Fig. 8 C, D). There was no apparent difference between those animals administered RMAC 8 and no evidence of podocyte detachment from the glomerular basement membrane in either group.

**Inhibition of cell adhesion using DE10**

Adhesion of 56/10 A1 cells to laminin and fibronectin was significantly inhibited by DE10 (Fig. 9), but binding to collagen I and collagen IV was not affected. Inhibition of binding with the positive control mAb against the α-subunit of VLA-3 (CD49c) was as predicted from published specificity of VLA-3. The only difference between DE10 and the mAb against VLA-3 was the inability of DE10 to inhibit binding to collagen I.

**DISCUSSION**

The mAb DE10 was made against recombinant human HB-EGF. It was found to be specific for human HB-EGF by ELISA, and reactivity with cells transfected with expression constructs for both human and rat HB-EGF cDNA. The antibody also inhibited function of recombinant human HB-EGF as assessed by inhibition of cell proliferation.

We have previously described an increase in HB-EGF mRNA and protein in glomerular epithelial cells (GECs) prior to the onset of proteinuria in both PAN and passive Heymann nephritis [7]. The function of increased HB-EGF in this location is unknown, but we proposed that it might contribute to the onset of proteinuria, either through stimulation of abortive mitogenesis of GEC with subsequent detachment from the GBM or binding of HB-EGF to the GBM, so altering its filtration properties. Therefore, it was surprising that administration of DE10 increased proteinuria early in the course of the PAN model, suggesting that up-regulation of HB-EGF was acting to decrease proteinuria. The findings did not appear to be due to complement-mediated injury to GEC because administration of cobra-venom factor did not affect the result. There are at least three potential explanations for these observations.

First, there is evidence that the recirculation of albumin in the glomerular filtrate through the proximal tubule may be considerable. It has been estimated that 1.8 mg/min of albumin per rat kidney may recirculate in this way (abstract; Eppel et al, *J Am Soc Nephrol* 9:337A, 1998). It is possible that blocking HB-EGF interferes with this pathway. However, this appears an unlikely explanation, because administration of DE10 had no effect on the level of proteinuria in the PAN model when administered at day 5 after induction. If the pathway was blocked, then this should increase proteinuria irrespective of the time of administration.

A second potential explanation relies on the ability of the transmembrane form of HB-EGF to inhibit apoptosis in epithelial cells. This observation was made by Takemura et al in the renal tubular epithelial cell line NRK 52E [15]. Since so-called “bare areas” of the GBM are reported to be the site of greatest passage of tracer molecules in PAN [16, 17], up-regulation of HB-EGF may prevent detachment of GEC because of cell injury, and thus reduce the bare areas. This explanation appears unlikely because we did not observe an increase in bare areas of the GBM in rats treated with DE10 compared with controls, although these could be distributed in a focal manner.

Finally, HB-EGF has been reported to associate with the integrin VLA-3 on the surface of various cell types. VLA-3 is the major integrin present on human GEC in
Fig. 9. Inhibition of adhesion of the human glomerular epithelial cell line 56/10 A1 to extracellular matrix constituents using monoclonal antibodies. Lane A represents wells coated with BSA in each panel. Lanes B–E were coated with collagen IV, collagen I, laminin, or fibronectin as indicated. Wells labeled B were treated with no mAb, well C with DE10 (anti-HB-EGF), well D with 11G5 (anti-CD49c), and well E with Ox34 as an isotype-matched irrelevant control. All panels show representative results of three to six separate experiments. *P < 0.005; **P < 0.001.

inhibiting HB-EGF worsens proteinuria

vivo, and there is increasing evidence that it is important in maintaining podocyte morphology through its contacts with actin microfilaments inside the cell. The function of the interaction between HB-EGF and VLA-3 is presently unknown. However, it is possible that HB-EGF modifies the adhesive properties of VLA-3 and helps to maintain podocyte morphology. In these studies, the adhesive role of HB-EGF is further strengthened by the demonstration that the inhibitory mAb DE10 reduced adhesion of the GEC line 56/10 A1 to fibronectin and laminin. Despite this finding in vitro, however, we were unable to demonstrate any difference in morphology between animals treated with DE10 or control antibody.

The albuminuria that occurred after administration of DE10 to normal rats was relatively slight and separates this mAb from some others that produce massive proteinuria. For example, a single intravenous injection of a mAb against the slit diaphragm protein nephrin caused massive proteinuria [18], as did a mAb directed against the active center of aminopeptidase A [19]. However, neither of these antibodies has been reported to worsen the proteinuria associated with the epithelial cell toxin PAN. We have interpreted this finding as evidence that HB-EGF limits the response to epithelial cell injury. This could occur either through the known ability of HB-EGF to increase cell survival in response to pro-apoptotic stimuli [15] or the adhesive properties of HB-EGF, demonstrated in this article. Although the albuminuria observed in response to injection of DE10 in normal glomeruli was minimal, it suggests that HB-EGF also has a role in maintaining the filtration barrier in normal glomeruli.

Finally, DE10 is the first mAb reported against HB-EGF. Its ability to bind both human and rat HB-EGF and to inhibit function suggests that it may be a useful reagent in defining the role of HB-EGF in kidney diseases and other conditions, such as atherosclerosis, wound healing, and cancer, where it has been identified as a potentially important growth factor.

ACKNOWLEDGMENTS

We acknowledge support from the National Health and Medical Research Council, the Baxter Extramural Grant Program, the St. Vincent’s Hospital Research Grants Endowment Fund, and the Australian Kidney Foundation. We also thank Mr. Julian Ophal, Department of Anatomical Pathology, who prepared the sections for electron microscopy. Parts of this work were presented at the American Society of Nephrology meeting in Philadelphia in October 1998 and were published in abstract form (J Am Soc Nephrol 9:500A, 1998).

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