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Human glomerular visceral epithelial cells synthesize a basal lamina collagen *in vitro*

(C3b receptors/3-hydroxyproline/mesangial cells/fibronectin)

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ABSTRACT **Isolated human glomeruli were digested with purified bacterial collagenase yielding epithelial cells. These cells grew to saturation density and did not become multilayered. They were identified as visceral glomerular epithelial cells by their morphologic appearance by phase and electron microscopy and by the presence of surface receptors for C3b. Neither Factor VIII antigen nor Fc receptors were observed. The glomerular epithelial cells synthesized a collagenous protein that was antigenically similar to human glomerular basal lamina. Proteins precipitated from visceral epithelial cell medium with affinity purified antibody against noncollagenous glomerular basal lamina antigens yielded a single collagenase labile protein that by sodium dodecyl sulfate/polyacrylamide gel electrophoresis migrated with an apparent M_r of 168,000 in the presence of reducing agents. Analysis of hydroxyproline isomers yielded a ratio of 3-hydroxyproline to total hydroxyproline of 0.17. Pepsin digestion yielded a disulfide-bonded multimer which, with reduction, migrated with an apparent M_r of 148,000. These data demonstrate that human glomerular visceral epithelial cells can be isolated and propagated *in vitro* and that they synthesize a collagen similar to that found *in vivo*.**

The glomerular basal lamina (GBL) is a part of the filtration barrier and functions to maintain the shape and elasticity of the glomerulus in the face of a high intraluminal pressure (1). Glomerular diseases leading to significant morbidity and mortality are associated with changes in the width, charge, and staining properties of the human GBL. The biochemical correlates of these changes, including the nature of the GBL biosynthetic subunits, their site(s) and synthesis, mechanism of degradation, and the factors regulating these processes, have not been eludicated.

Based on its role as the primary supportive structure of the glomerulus and its content of hydroxyproline, the GBL has long been thought to contain a collagenous protein(s) (2), but isolation of an intact collagen has proven difficult. Extraction with aqueous and organic solvents yielded multiple proteins with varying molecular weight and hydroxyproline content (3). This was postulated to be due to extensive covalent crosslinking and partial proteolysis during the isolation process (3). Other investigators, taking advantage of the resistance of collagens to proteases, found that limited enzymatic digestion with pepsin solubilized helical collagenous proteins (4, 5) that are unique in amino acid sequence (6) and composition (4). Amino acid analysis of the pepsin-resistant proteins were substantially different from that which would be predicted from analysis of the intact human GBL.

These data indicated that intact molecules could not readily be isolated from human GBL and led to studies of basal lamina collagen synthesis by suspensions of glomeruli *in vitro* (7, 8). Although significant amounts of radiolabeled collagen were synthesized, its site of synthesis was not defined. The human GBL, a morphologically homogeneous structure, has three associated cell types, each of which has an adjacent basal lamina in other tissues. The glomerular visceral epithelial cells lie on a basal lamina that invests the capillary loops and the adjacent mesangial regions. Attempts to demonstrate that visceral epithelial cells synthesized basal lamina components *in vivo*, normally or after injury, have made use of the deposition of dense granules in GBL after administration of silver nitrate. However, the presence of granules on the basal lamina appeared to induce changes in synthesis or turnover of the normal GBL (9). The mesangial cells resemble smooth muscle cells that are surrounded by a basal lamina *in vivo* and synthesize types I, III, and A and B collagens *in vitro* (10). Vascular endothelial cells similarly are associated with a basal lamina *in vivo* and have been shown to synthesize a basal lamina collagen *in vitro* (11). Thus, any one or all of the glomerular cells may have been synthesizing the basal lamina collagen observed in organ culture. Preliminary studies in this laboratory provided evidence that individual glomerular cells may synthesize collagens of different genetic type with different posttranslational features (12). In this study we have isolated, identified, and propagated human glomerular visceral epithelial cells and have demonstrated the synthesis of a procollagen-like protein resembling the basal lamina collagen found in human glomeruli.

MATERIALS AND METHODS

Isolation of Glomerular Epithelial Cells. Isolated glomeruli (13) were incubated with purified bacterial collagenase (Worthington CLSPA), 750 units/ml, in complete Waymouth's medium for 30 min at 37°C to remove visceral epithelial cells (14). The glomerular suspension was allowed to settle to remove intact glomeruli. Partially digested glomerular segments were separated from free cells on stainless steel screens (350 mesh). The glomeruli and free cells were examined by phase and transmission electron microscopy to assure complete separation. The isolated cells were propagated in complete Waymouth's medium supplemented with 20% fetal calf serum (FCS) or pooled human serum (PHS).

Assessment of Fc and C3b Receptors. Assays for specific receptors for the Fc portion of IgG or C3b were performed by standard immunocytoadherence techniques (15). A cell was considered rosette positive if it possessed five or more adherent sheep erythrocytes. The positive control tested was human peripheral monocytes and the negative control was cultured human fibroblasts maintained in either 20% FCS or 20% PHS. In some experiments cells were maintained in medium containing 20% FCS but were washed with medium containing 20% PHS 2 hr prior to assay.

Assessment of Phagocytosis. Phagocytosis was assessed by using india ink particles (Pellikan C11/1431a, Gunther Wagner) or latex microspheres (1.017 μm in diameter). Cultures were incubated with the particles for 2 hr and then washed with

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Abbreviations: GBL, glomerular basal lamina; $NaDodSO_4$, sodium dodecyl sulfate; FCS, fetal calf serum; PHS, pooled human serum.

medium. The cell layers were examined for intracellular particles by phase microscopy.

Antibody Preparation. Human GBL was prepared from kidneys as described (16). Lyophilized GBL (100 mg) in complete Freund's adjuvant was injected every 2 weeks into each of two sheep. After 7 weeks, both sheep developed renal failure and biopsy of their kidneys disclosed crescentic glomerulonephritis with linear deposition of sheep IgG on the GBL.

Specific anti-human GBL antibody was prepared from nephritic sheep IgG by affinity chromatography. IgG was eluted at low pH from CNBr-activated Sepharose (Pharmacia) coupled with human GBL antigens solubilized by collagenase digestion (17). Amino acid analysis of these antigens after digestion revealed 9% residual hydroxyproline. The eluted IgG was adsorbed against human types I and III collagen purified from human skin (18) and human serum proteins. Serial dilutions of affinity-purified antibody were examined for staining of human GBL by indirect fluorescence microscopy.

Affinity-purified rabbit anti-human Factor VIII antibody was provided by Richard Counts and Gottfried Schmer. After brief exposure to ice-cold 50% methanol, glomerular epithelial cells were incubated with this antibody and examined by indirect immunofluorescence. Human umbilical vein endothelial cell cultures served as positive controls (19).

Affinity-purified rabbit anti-cold-insoluble globulin was a gift of G. Balian.

Labeling of Cell Proteins. Glomerular cell cultures were incubated for 2 hr in serum- and proline-free Waymouth's medium supplemented with β-aminoproprionitrile and sodium ascorbate (50 μg/ml). Fresh medium containing 20 μCi (1 Ci = 3.7×10^{10} becquerels) of L-[2,3-^{3}H]proline per ml or 7 μCi of L-[^{14}C]proline per ml (New England Nuclear) was added. After 24 hr the medium was decanted and protease inhibitors were added to achieve final concentrations of 25 mM EDTA, 10 mM *N*-ethylmaleimide, and 1 mM phenylmethylsulfonylfluoride.

^{3}H-Labeled Amino Acid Analysis. Acid hydrolysates of culture medium or cell layer were chromatographed on a 58 × 0.9 cm column containing a sulfonated polystyrene resin (Beckman UR-30) and were eluted with 0.2 M sodium citrate (pH 3.0) at ambient temperatures. 3-Hydroxyproline and 4-hydroxyproline were resolved from proline by 78 and 60 ml, respectively. Elution volumes were confirmed with acid hydrolysates of human GBL. Fractions were collected, and ^{3}H and ^{14}C were measured by using standard liquid scintillation counting techniques. Medium from cells labeled with L-[^{14}C]proline and L-[^{3}H]proline were analyzed to quantitate the loss of ^{3}H from the 3 position of proline during biological oxidation. All values reported have been corrected for this loss.

Enzymatic Digestion. Lyophilized glomerular cell or fibroblast media were dissolved in 0.5 M acetic acid at 4°C. Pepsin was added in an enzyme to substrate ratio of 1:100 (wt/wt). The samples were incubated for 16 hr at 4°C, lyophilized, and analyzed by sodium dodecyl sulfate ($NaDodSO_4$)-/polyacrylamide gel electrophoresis. Fibroblast procollagens were completely converted to constituent α1 and α2 chains. Lyophilized radiolabeled glomerular cell medium was digested with purified bacterial collagenase (Advanced Biofactures, Lynbrook, NY) in 0.1 M Hepes buffer (pH 7.6) containing 10 mM *N*-ethylmaleimide, 5 mM $CaCl_2$, and 0.1 mM phenylmethylsulfonylfluoride at 37°C for 4 hr. There was insignificant release (less than 1%) of radioactivity from radiolabeled bovine serum albumin under these conditions. After precipitation in cold 10% trichloroacetic acid, the protein was analyzed by $NaDodSO_4$ gel electrophoresis.

Immune Precipitation. Affinity-purified sheep anti-human GBL was added in excess to 2 ml of culture medium. After incubation for 1 hr at room temperature, the sheep IgG was quantitatively precipitated by the addition of rabbit anti-sheep IgG and the precipitate was washed with cold saline. The pellets were digested with collagenase or pepsin and analyzed by $NaDodSO_4$ gel electrophoresis. Normal sheep IgG failed to precipitate radiolabeled protein.

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Proteins from human glomerular epithelial cell and fibroblast culture medium were separated by electrophoresis on 5% $NaDodSO_4$/polyacrylamide slab gels by using a discontinuous buffer technique (20). Samples were dissolved by boiling for 2 min in 2% $NaDodSO_4$ sample buffer or in sample buffer containing 50 mM dithiothreitol. After electrophoresis, radioautofluorograms were prepared (21). Molecular weights were calculated from fibrous protein internal standards.

RESULTS

Isolation and Identification of Glomerular Epithelial Cells *In Vitro*. Epithelial cells from freshly isolated human glomeruli were studied by phase and electron microscopy. Electron micrographs of glomeruli incubated for 30 min in bacterial collagenase demonstrated denudation of the peripheral basal lamina. The cells isolated from the digestion supernate remained a confluent monolayer until sacrificed after 5 weeks without passage. By phase microscopy the monolayer consisted of a homogeneous population of angular cells, which were epithelial in appearance (Fig. 1 *top*). Electron microscopic examination disclosed a monolayer of homogeneous cells with abundant cytoskeletal filaments organized into cell junctional complexes. No peripheral dense bodies or Weibel–Palade bodies were seen. Profiles of rough endoplasmic reticulum were sparse when compared with human fibroblasts or vascular smooth muscle cells *in vitro*.

Because C3b receptors alone were found on glomerular visceral epithelial cells *in vivo* (22) and *in vitro* (23, 24), cultures were examined for these receptors. Fc receptors and phagocytic capacity were assayed to detect potentially contaminating macrophages (25). Glomerular epithelial cells maintained in medium supplemented with FCS bound the complement-coated sheep erythrocytes but failed to do so when the erythrocytes were coated with IgG or IgM (Fig. 1 *middle*). Human peripheral blood monocytes demonstrated both C3b and Fc receptors by this technique. C3b receptors were not detectable on glomerular epithelial cells when PHS was used to propagate these cells. In addition, cells plated and maintained in FCS and then washed with PHS 2 hr prior to assay failed to demonstrate C3b receptors. Human fibroblasts failed to demonstrate either receptor, regardless of medium. Glomerular epithelial cells failed to phagocytose latex or india ink particles, whereas peripheral blood monocytes actively ingested these particles.

Cultures containing glomerular epithelial cells were examined by indirect immunofluorescence for Factor VIII antigen, a marker specific for vascular endothelial cells (19). The cytoplasm of control human umbilical cord vein endothelium was strongly positive, whereas none of the cells in the glomerular epithelial layer demonstrated this antigen. Thus, vascular endothelial cells were not contaminants of the glomerular epithelial cell monolayers.

Hydroxyproline Analysis. Incorporation of [^{3}H]hydroxyproline into nondialyzable protein by glomerular epithelial cells was linear between 2 and 24 hr. Cultures were pulse-labeled with [^{3}H]proline for 2 hr followed by a 22-hr chase with fresh medium containing excess unlabeled proline to assess the partitioning of newly synthesized collagen between medium and cell layer. Seventy percent of the total radiolabeled hydroxyproline was found in the culture medium. The ratio of hydroxyproline to proline in the culture medium was 0.086 ±

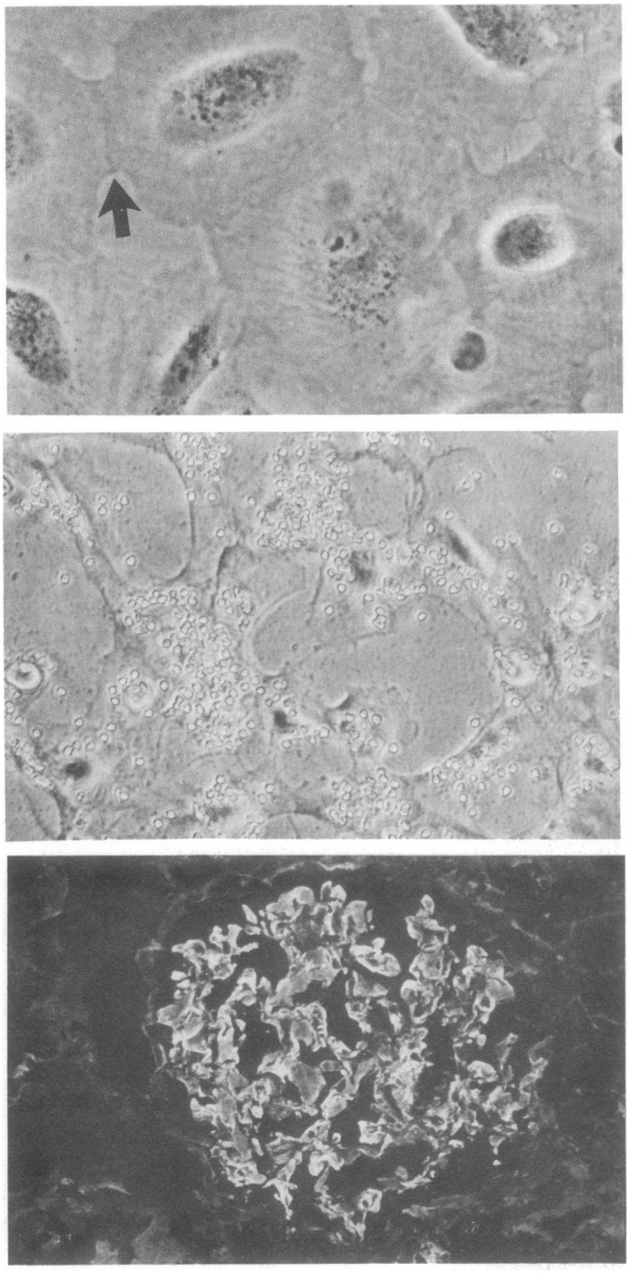

FIG. 1. (*Top*) Phase contrast photomicrograph of glomerular epithelial cell culture. Large polygonal cells show areas of close contact (arrow) which by electron microscopy contain desmosomes and gap junctions. (×280.) (*Middle*) Phase contrast photomicrograph of glomerular epithelial cells after incubation with sheep erythrocytes coated with subagglutinating amounts of IgM and complement. Note the high density of erythrocytes over the cell cytoplasm compared with the culture surface. No difference in the density of erythrocytes over cytoplasm or plastic was observed if the erythrocytes were coated with IgG or IgM alone. (×280.) (*Bottom*) Fluorescence photomicrograph of normal human kidney cortex. A frozen section was incubated in a 1:50 dilution of sheep anti-human GBL IgG affinity-purified against noncollagenous GBL antigen and adsorbed against human interstitial collagens and serum proteins. A 1:10 dilution of fluorescein-conjugated rabbit anti-sheep IgG adsorbed against human serum was used to demonstrate specific antibody. Note linear staining of GBL and relative absence of staining of tubular basal lamina and Bowman's capsule. (×360.)

0.025 ($\bar{X}$ ± SD). This was 5 times greater than in the cell layer, indicating that the medium was selectively enriched in collagenous proteins. The ratio of 3-hydroxyproline to total hydroxyproline in both the medium and cell layer was 0.177 ± 0.012. Analyses of human fibroblast culture medium revealed ratios of hydroxyproline to proline and 3-hydroxyproline to total hydroxyproline of 0.286 ± 0.058 and 0.012 ± 0.004, respectively. Both values were significantly different from those of glomerular epithelial cells ($P < 0.001$).

Electrophoretic Behavior of Medium Proteins. The nature of the collagenous protein synthesized by glomerular cells *in vitro* was investigated by $NaDodSO_4$ gel electrophoresis after digestion with pepsin at 4°C or with purified bacterial collagenase (Fig. 2). Control fibroblast medium was similarly processed. Medium from glomerular epithelial cell cultures revealed a single collagenase-labile protein with an apparent M_r of 168,000 in the presence of reducing agents (Fig. 2, lanes C and D). This molecule existed as a high M_r multimer when reducing agent was omitted. Pepsin digestion of glomerular epithelial cell medium yielded a single major component with an apparent M_r of 148,000 after reduction (Fig. 2, lane E). Occasionally this band was resolved into a doublet; however, the ratio of the two constituents was variable. The pepsin-resistant molecules migrated as an apparent trimer in the absence of a reducing agent. Under these conditions collagenous proteins from fibroblast medium behaved quite differently than those from glomerular cell medium (Fig. 2, lanes A and B).

The predominant proline-labeled protein synthesized by glomerular epithelial cells (54% by radiodensitometry) migrated as a dimer in the absence of reducing agent, and after reduction migrated as a polydisperse protein with an apparent M_r of 240,000. This protein was collagenase resistant, pepsin sensitive, and precipitable with affinity-purified anti-cold-insoluble globulin. It thus resembled fibronectin (26).

Precipitation of Human GBL Antigens Synthesized *In Vitro*. IgG from sheep immunized with human GBL was affinity purified with collagenase-solubilized human GBL antigens and adsorbed against human skin types I and III collagen

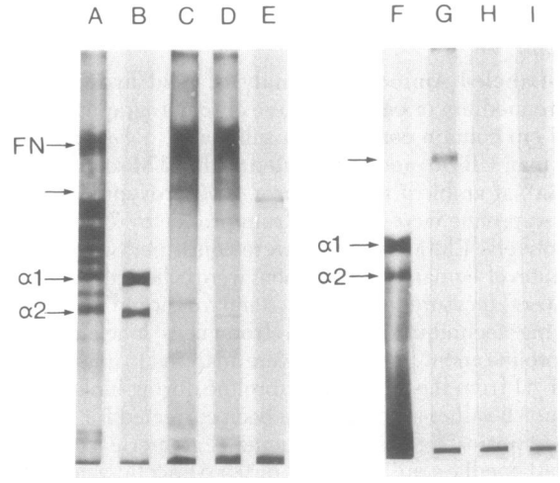

FIG. 2. Radioautofluorogram of medium proteins. All samples were reduced with dithiothreitol prior to electrophoresis. Lane A: Fibroblast medium demonstrating procollagen species and fibronectin (FN). Lane B: Fibroblast medium after digestion with pepsin. Lane C: Visceral epithelial cell medium. Note the presence of fibronectin (M_r 240,000) and a protein of M_r 168,000 (arrow). Lane D: Visceral epithelial cell medium. Treatment with bacterial collagenase degrades the M_r 168,000 protein. Lane E: Visceral epithelial cell medium. Treatment with pepsin results in a single resistant protein of M_r 148,000. Frequently a second protein was resolved, suggesting a doublet. Lane F: Fibroblast medium treated with pepsin as internal standard for immunoprecipitate. Lane G: Immunoprecipitate of visceral epithelial cell medium with sheep anti-human GBL IgG affinity purified against noncollagenous antigens selectively enriches the M_r 168,000 protein (arrow). Lane H: Immunoprecipitate of visceral epithelial cell medium. Digestion with collagenase degrades the M_r 168,000 protein. Lane I: Immunoprecipitate of visceral epithelial cell medium. Treatment with pepsin yields a protein similar to that observed in pepsin-digested medium.

and human serum proteins. Frozen sections of normal human kidney were examined by indirect immunofluorescence with this antibody as well as IgG from normal sheep serum and IgG from nephritic sheep serum, which was not bound by the affinity column. The affinity-purified and adsorbed sheep anti-GBL IgG linearly stained the human GBL in frozen sections of normal kidney cortex (Fig. 1 *bottom*). The IgG that was not bound to the affinity column and IgG from normal sheep did not stain these structures.

Radiolabeled medium proteins of glomerular epithelial cells were precipitated with specific antibody to GBL and were hydrolyzed and analyzed. The ratio of 3-hydroxyproline to total hydroxyproline was 0.170, nearly identical to the starting material. The ratio of hydroxyproline to proline was 0.35. Similar antibody-precipitated medium was digested with pepsin or bacterial collagenase. Electrophoresis of the precipitate in the presence of dithiothreitol revealed a collagenase-labile protein with an electrophoretic mobility identical to that observed in glomerular epithelial cell medium (Fig. 2). When treated with pepsin, this M_r 168,000 species converted to an apparent M_r of 148,000. Thus, the affinity-purified antibody precipitated a collagenous protein that closely resembled that observed in the whole medium.

DISCUSSION

The difficulty in solubilizing intact collagen molecules from glomeruli isolated from renal cortex has led investigators to study collagen synthesis by glomeruli *in vitro*. Suspensions of intact glomeruli synthesized hydroxyproline and glycosylated hydroxylysine (7, 8), but the cellular site(s) of synthesis was not identified. Explanted glomeruli gave rise to several morphologically distinct cells (13). These cultures of mixed glomerular cells synthesized collagenous proteins with posttranslational features resembling GBL (12). In such cultures it was not determined which glomerular cells synthesized a basal lamina collagen, because the constituent cells were not isolated and identified and the collagen they synthesized was not shown to be related to that found in the GBL *in vivo*.

Collagenase digestion of isolated glomeruli released visceral epithelial cells from their matrix (14). The epithelial cells formed a confluent monolayer, whereas cultivation of the denuded glomerular fragments resulted in a population of smooth muscle-like cells which became multilayered (14). The persistence of markers, similar to those found *in vivo*, was used to precisely identify cells *in vitro*. C3b receptors have been localized to glomeruli *in vivo* (22) and were shown to reside on the visceral glomerular epithelial cells of human glomeruli explanted *in vitro* (23). In addition, rat glomerular epithelial cells were found to retain C3b receptors over prolonged periods in culture (24). In the current study we found that human glomerular epithelial cells possessed C3b receptors on their surface for prolonged periods *in vitro* but that they were masked by exposure to human serum. This may have been due to the presence of C3 fragments in pooled human serum, which have been shown to inhibit such rosettes formed with murine (27) and human (28) lymphocytes. Human glomerular epithelial cells were distinguished from endothelium by the absence of cytoplasmic Factor VIII (19), from macrophages by the absence of Fc receptors or phagocytosis of particles (25), and from mesangial cells by the presence of C3b receptors and by their failure to synthesize collagen types I, III, and B (unpublished observations). Thus, the epithelial cells isolated from human glomeruli in this study were visceral epithelial cells.

The ability of glomerular visceral epithelial cells to synthesize collagenous proteins was assessed by analysis of nondialyzable hydroxyproline in radiolabeled culture medium and cell layer. Seventy percent of the nondialyzable hydroxyproline was found in the medium after a 22-hr chase. A similar observation has been made for fibroblasts (29) and smooth muscle cells (30). The ratio of hydroxyproline to proline has been used to approximate the relative amount of collagen synthesized by cells in culture (31). Visceral epithelial cells hydroxylate 10% of the nondialyzable proline found in the medium. This value was substantially less than that observed for vascular smooth muscle cells (42%) and fibroblasts (28%) but was significantly greater than that observed for human umbilical vein endothelium (4%) when these cells were labeled in identical conditions (unpublished observations). Thus, the glomerular visceral epithelial cells synthesized and secreted a collagenous protein. Because the amino acid composition of basal lamina collagens has been shown to be distinctive, amino acid analyses were used to assess whether a basal lamina collagen was synthesized by glomerular epithelial cells. Compared with interstitial collagens, basal lamina collagen has been reported to have relatively less alanine and arginine and more cysteine. In addition, a high degree of hydroxylation of lysine and proline, glycosylation of hydroxylysine, and hydroxylation of proline in the 3 position have been shown to be characteristic of collagens isolated from several sources of basal lamina (32). We found that isolated visceral epithelial cells synthesized a collagen that had a ratio of 3-hydroxyproline to total hydroxyproline of 0.177. This value was similar to that reported for human GBL (4) and that reported by Scheinman *et al.* (33) for "circular glomerular cells." In contrast to the mixed cell population obtained from explanted glomeruli (14), this ratio was stable over a 5-week period in culture. These data were in agreement with the light microscopic conclusion that the cultures were a homogeneous population of epithelial cells. Although posttranslational features were useful in identifying basal lamina collagens, they were not specific. For instance, it has recently been shown that $[\alpha 1(I)]_3$ synthesized by amniotic fluid cells in culture shares many of the posttranslational features of a basal lamina collagen (34). In contrast, A and B collagens isolated from placental membranes and thought to be basal lamina in origin had a low 3-hydroxyproline content (35, 36).

A method used to establish that the collagenous protein synthesized by visceral epithelial cells was a basal lamina component was to demonstrate that it shared antigenic determinants with GBL. Antibody that was affinity purified against collagenase-resistant human GBL antigens stained the human GBL in normal kidney cortex in a linear fashion. This antibody precipitated radiolabeled visceral epithelial cell medium proteins, which had a ratio of hydroxyproline isomers (0.17) similar to that found in the whole medium. The ratio of total hydroxyproline to proline was 0.35. This value was in good agreement with that observed for the collagen found in the medium of parietal yolk sac endoderm cultures (37) but was significantly less than the ratio of 0.70 which had been reported for the collagen isolated by pepsin digestion of isolated GBL (4). The low ratio may have been due to the secretion of a partially hydroxylated collagen or to the presence of noncollagenous protein, either covalently linked to the collagen or coprecipitated with this molecule.

The collagen observed in the medium of glomerular visceral epithelial cells had an apparent M_r of 168,000 after reduction and 148,000 after pepsin digestion. Both proteins migrated as higher M_r multimers in the absence of reducing agent, suggesting that they possessed interchain disulfide bonds. These data indicate that the visceral epithelial cells synthesize a procollagen-like molecule (38, 39). Precipitation of medium with antibody to noncollagenous human GBL antigens resulted in substantial purification on this protein, suggesting that procollagen antigens may be found in the normal human GBL. A similar protein has been described for other basal lamina col-

lagen-synthesizing systems, including explanted glomeruli from malignant hypertensive kidneys (40), rat parietal yolk sac endoderm (37), and human amniotic fluid cells (41). In the latter case the biosynthetic product has been purified; its amino acid composition resembled type IV collagen and cyanogen bromide peptides were distinct from those of α1(I), α1(II), α1(III), and α2.

It has been suggested that type IV collagen exists in tissues with covalently associated noncollagenous moieties (6, 42–45). Proteolytic digestion of basal lamina from diverse sources (4, 5, 42–46) has resulted in the solubilization of disulfide-crosslinked collagen chains with a M_r between α and β chains of collagen. Analyses of some of these proteins revealed them to be substantially enriched in amino acids found in nonhelical procollagen domains (42). Indeed, these residues and heteropolysaccharides could be found in a single cyanogen bromide peptide (6). Examination of solutions of these molecules and segment-long-spacing crystallites by electron microscopy revealed a globular structure at one end of the collagen molecule (43, 44). The globular moiety was rendered digestible by pepsin after reduction and alkylation in nondenaturing solutions. These data have supported the suggestion that type IV collagen exists in tissues as a procollagen (42). The fact that pepsin digestion of glomerular visceral epithelial cell medium and of the immunoprecipitate yielded a protein analogous to that found in tissues suggested that this was a fundamental property of the collagen and was not the result of organization of this protein with others in the extracellular matrix. The role of disulfide crosslinks in maintaining a pepsin-resistant conformation and the relation of this molecule to those found in tissue are of interest.

The demonstration that glomerular visceral epithelial cells synthesize a component of the GBL is of considerable biological significance because basal lamina abnormalities are associated with most forms of chronic glomerular disease. The ability to obtain and define homogeneous cultures of these cells makes it feasible to investigate factors influencing such cell functions as basal lamina synthesis. The further study of the basal lamina components that they synthesize may provide approaches to the characterization of various inflammatory and genetic diseases which heretofore have been characterized by morphologic means.

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