Preservation of Mesangium and Immunohistochemically Defined Antigens in Glomerular Basement Membrane Isolated by Detergent Extraction

MARK T. HOUSER, JON I. SCHEINMAN, JOHN BASGEN, MICHAEL W. STEFFES, and ALFRED F. MICHAEL, *Departments of Pediatrics, Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota 55455*

ABSTRACT To define the characteristics of isolated glomerular basement membrane (GBM), immunohistochemical and morphometric analyses have been carried out on rat and human tissues. Site-specific arrays of antigens were identified in detergent-isolated GBM in a distribution similar to that observed in intact kidney. In the human, fibronectin, procollagen IV, and collagen V were observed along the internal aspect of GBM continuous with antigenic sites in the mesangium. Another array of antigens was identified in the GBM but not within the mesangium—Goodpasture's antigen, bovine lens capsule type IV collagen, and amyloid P component. In addition, sites reactive with rabbit antiserum to laminin were present on both sides of the lamina densa as well as within the mesangial region. Actomyosin, a presumed mesangial cell antigen persisted in the mesangium of isolated GBM. Mesangial matrix was identified in detergent-isolated GBM in an amount equivalent to that present in intact glomeruli. Sonicated GBM contained the same antigens but it was not possible to quantitate the amount of mesangial material by immunofluorescence or morphometric analysis. The thickness of the lamina densa was greater in sonicated and detergent-treated rat GBM preparations than in native rat kidney. These studies demonstrated that isolated GBM is heterogeneous with respect to its antigenic constituents and in addition contains mesangial matrix, which is morphologically and immunohistochemically distinct from peripheral GBM.

INTRODUCTION

The glomerular basement membrane (GBM),[1] interposed between the capillary endothelium and epithelium, plays a decisive role in regulating the movement of macromolecules into the urinary space. The relative ease with which GBM has been isolated by ultrasonic disruption and, more recently, by detergent extraction has led to numerous studies of its biochemical composition and structure (1–7). Controversy over the structural relationship of collagen and noncollagen moieties has not been resolved (6), although genetically distinct species of basement membrane collagen have been characterized (7). Distinct antigenic species of biochemically similar collagens have been suggested by our recent immunohistochemical studies (8).

Recently, discrete collagen and glycoprotein antigens have been demonstrated in site-specific arrays along the glomerular capillary wall and within the mesangium (8–18). It has not been clear whether these antigens are intrinsic components of the GBM that correspond to isolated moieties or are altered and perhaps lost during isolation by either sonication or detergent extraction. However, amyloid P component has been identified in collagenase-digested preparations of GBM (18). Our current studies demonstrate that isolated GBM is heterogeneous, containing arrays of collagen and glycoprotein antigens similar in distribution to those present in intact tissue, and mesangial material in a proportion similar to that present in intact glomeruli. The antibody probes used in the study were selected on the basis of known reactivity with glomerular structures in tissue sections.

METHODS

Isolation of glomeruli and glomerular basement membrane. Rat kidneys were obtained from a group of 15 Sprague-Dawley males weighing 265–290 g after killing by

Received for publication 29 June 1981 and in revised form 7 January 1982.

Address reprint requests to Dr. Michael.

[1] *Abbreviations used in this paper*: BLC IV, type IV collagen from bovine lens capsule; DL, double linear; ELISA, enzyme linked immunoassay; FITC, fluorescein-isothiocyanate; GBM, glomerular basement membrane; GP, Goodpasture; I, internal; SL, single linear.

cervical dislocation. Human kidneys were obtained at autopsy from two male patients (age 25 and 40 yr) dying acutely from accidental causes. Glomeruli were isolated at 4°C using stainless steel sieves and GBM was isolated from glomeruli by either sonication for 2–3 min at 4°C in 1 M sodium chloride (19, 20) or by detergent-extraction technique of Meezan et al. (5) using hypotonic cell lysis, incubation with DNAse, extraction with desoxychloate and extensive washing over a nylon screen (pore size 20 μm) with distilled water.

Immunohistochemical studies. Cortical slices of human and rat kidneys were snap frozen in isopentane precooled in liquid nitrogen and were processed for immunohistochemical analysis as previously described (8, 11, 12). GBM preparations isolated by either detergent-extraction or sonication were dispersed in cryoform (Damon/IEC Division, Needham Heights, MA) within microfuge tubes (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) by low speed centrifugation (100 *g* for 5 min) and frozen in liquid nitrogen. The tubes were mechanically broken and the frozen plug placed in isopentane precooled in liquid nitrogen and stored at −70°C until processed for immunofluorescence. High-resolution phase contrast and indirect epifluorescence were used to localize specific antigens in rat and human preparations as previously described (8, 11, 12).

Affinity-purified rabbit antibodies with specificity for the following antigens were used: human placental type V ($\alpha A\alpha B$) collagen (antibody reacting only with the combined $\alpha A\alpha B$ collagen, but not the denatured α-chains, suggesting a conformational determinant (8); type IV pro-collagen (P-IV) derived from the EHS mouse sarcoma (8); and laminin (11). These antibodies were cross-absorbed with the other antigens and types I and III collagen, and were nonreactive with other enzyme-linked antigens by immunoassay (ELISA) (21). These antibodies were provided by Dr. J. M. Foidart (Liege, Belgium) and Dr. G. R. Martin (National Institute of Dental Research, Bethesda, MD). Affinity-purified rabbit antibody to fibronectin was nonreactive with laminin and P-IV on ELISA, and was provided by Dr. Leo Furcht (University of Minnesota). Anti-serum to the type IV collagen from bovine lens capsule (BLC IV) was provided by Dr. N. A. Kefalides (Philadelphia, PA). This antibody was nonreactive with types I and III collagen by radioimmunoassay (22), and clearly differed in tissue distribution from the P-IV antibody (8). Rabbit antiserum to amyloid P component was provided by Dr. Merrill D. Benson (Indianapolis, IN) (23, 24).

Uterine smooth muscle actomyosin was characterized by ATPase activity and gel electrophoresis and the antiserum by immunodiffusion and absorption (9, 25). These antibodies and antisera were localized with fluorescein-isothiocyanate-labeled (FITC) goat anti-rabbit IgG (N. L. Cappel Laboratories, Cochranville, PA). The eluted Goodpasture's (GP) antibody, which did not react with laminin or P-IV (10), was applied and localized with FITC rabbit anti-human IgG as previously reported (12). Sections were also stained with FITC-labeled goat anti-human albumin (26). Terms used to describe the immunofluorescence patterns include: double linear (DL), two discrete lines along the peripheral GBM; single linear (SL), linear staining within the peripheral GBM; internal (I), linear staining along the internal aspect of the peripheral GBM, which merges with antigenic sites in the mesangium.

Morphometric analysis. Cortical slices from five randomly selected rat kidneys, isolated glomeruli, and GBM were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, and processed for electron microscopy as previously described (27). GBM thickness was measured by the orthogonal intercept method of Jensen et al. (28). The total GBM thickness in intact kidney was measured as the distance from the endothelial cell to the epithelial cell. The central electron dense material in these sections defines the thickness of the lamina densa; this term was also applied to electron dense material in isolated GBM. The proportions (fractional volumes) of GBM and mesangial matrix were assessed on random sections of intact kidney, isolated glomeruli, and isolated GBM using a grid and point counting technique; the mesangium and GBM were defined as previously described (29). The data are presented as the proportion (or fractional volume) of points falling on one component compared to the total number of points falling on all three. At least three and usually five to seven glomeruli or zones were examined in these analyses. Values are expressed as mean±SD and probabilities were calculated using the Student's *t* test.

RESULTS

Morphometric studies. Electron microscopy of rat GBM preparations isolated by detergent extraction

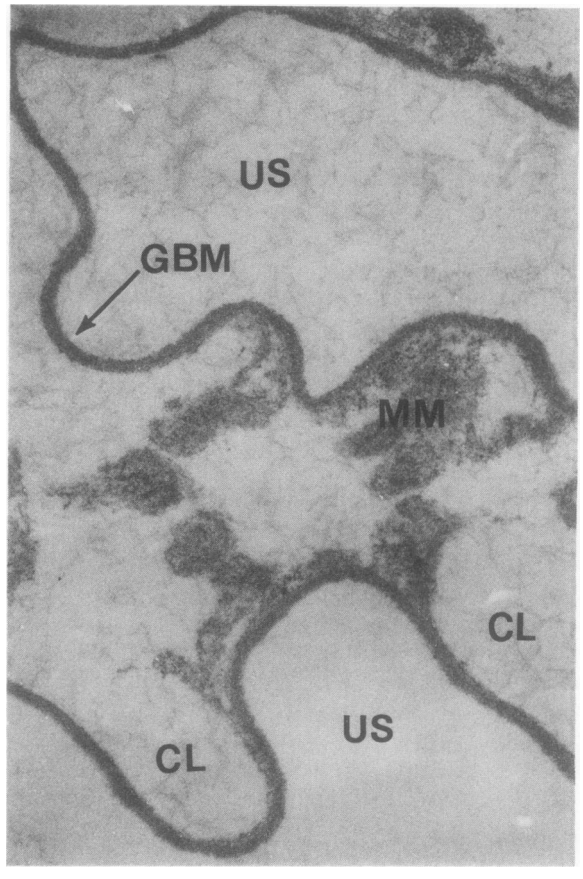

FIGURE 1 Electron micrograph of rat GBM isolated by detergent extraction (×13,000). Note the significant inclusion of mesangial matrix material. Abbreviations: US, urinary space; MM, mesangial matrix; CL, capillary lumen.

demonstrates this material to be ultrastructurally and spatially intact, free of cell membranes, and containing GBM as well as recognizable electron dense matrix material in intact mesangial zones (Fig. 1). The thickness of the rat lamina densa (mean±SD–85±5 nm) was similar to that in isolated glomeruli (88±4 nm), but was significantly increased in GBM derived from sonication (104±3 nm, $P < 0.001$) or detergent-extraction (93±5 nm, $P < 0.05$). The mesangial matrix constituted 39±6% of the total (matrix plus GBM) in both isolated rat glomeruli and GBM isolated by detergent extraction; this percentage was greater than that observed in intact kidney (24±4; $P < 0.001$).

Immunofluorescence microscopy. An analysis of the antigens present within human glomeruli and isolated GBM is given in Table I. These results are a composite of the findings in both human kidneys. In material isolated by detergent extraction as well as in glomeruli of intact tissue actomyosin was localized almost exclusively in the mesangium (Fig. 2A, B). P-IV, $\alpha A \alpha B$, and fibronectin were present in the mesangium, and the inner aspect of the GBM (Fig. 2C, D, Figs. 3 and 4). The location was appreciated by visually alternating phase-contrast and epifluorescence or by superimposing the images photographically, revealing that the linear immunofluorescence was along the internal aspect of the phase-dense image of the GBM. In contrast, the BLC-IV localized, in both intact tissue and isolated material, to the full thickness of the GBM as demonstrated by superimposition of the linear fluorescence with the phase-dense image of the GBM (Fig. 2E, F). GP antigen and amyloid P component were present in a SL pattern in the GBM but

TABLE I
Immunohistochemical Analysis of Antigens in Sections of Normal Human Kidney and GBM Isolated by Detergent Extraction

	Intensity and distribution of antigens as defined by immunofluorescence‡			
	Normal kidney		GBM isolated by detergents	
Antigens*	GBM	Mesangium	GBM	Mesangium
Collagens				
P-IV	1+ (I)	2–3+	1–2+ (I)	3+
IV (BLC)	1–2+ (SL)	tr	3+ (SL)	tr
V (AB)	1+ (I)	2+	1–2+ (I)	3+
Fibronectin	1+ (I)	2–3+	2+ (I)	2–3+
Laminin	2+ (DL)	1+	2–3+ (DL)	1+
Actomyosin	tr (I)	1–2+	1+ (I)	2–3+
GP	2+ (SL)	0	2–3+ (SL)	0
Amyloid-P	2+ (SL)	0	3+ (SL)	0

* P-IV, procollagen type IV; IV (BLC), bovine lens capsule type IV collagen.
‡ Intensity of immunofluorescence arbitrarily graded 0, tr, 1+, 2+, 3+. Localization along GBM is described as DL, SL, and I.

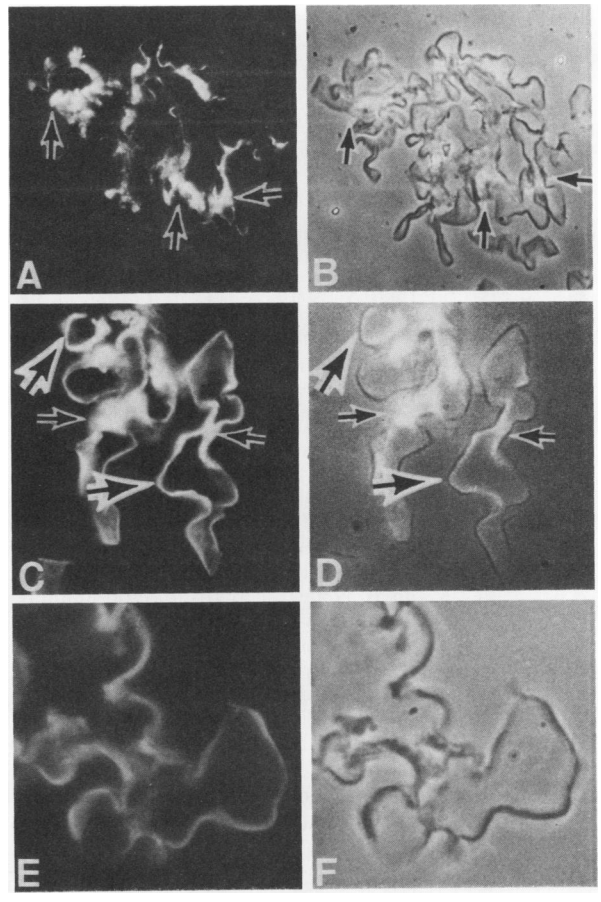

FIGURE 2 Antigens in human GBM isolated by detergent extraction. A, B: Actomyosin is present within the mesangium as seen by immunofluorescence alone (A) or in the same section with phase-contrast microscopy (B). Small arrows denote the mesangial region (×400). C, D: P-IV is observed along the GBM (large arrows) and within the mesangial region (small arrows) as seen by immunofluorescence alone (C) or in the same section in combination with phase-contrast microscopy (D). The latter demonstrates the linear fluorescence to be along the internal aspect of the phase-dense image of the GBM (×700). E, F: BLC (IV) is present along the full width of the GBM as shown by immunofluorescence (E); note that the fluorescent image has the same configuration as the phase dense line of the GBM as demonstrated by phase-contrast microscopy alone (F) (×700). Combined immunofluorescence-phase contrast is not illustrated since the fluorescence juxtaposes the phase-density; to illustrate the latter a higher transmitted light intensity is necessary, and this obscures the reflected-fluorescent image.

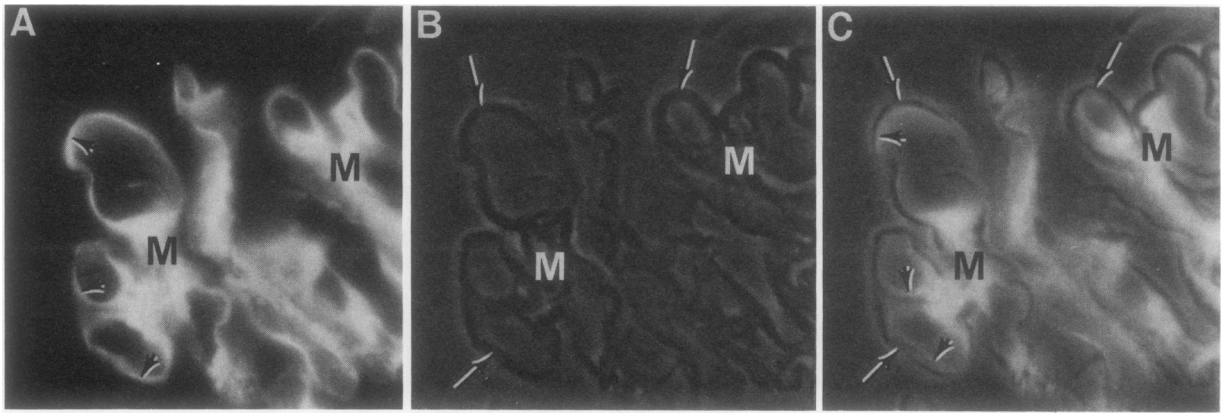

FIGURE 3 Photomicrograph of a segment of a glomerulus. (A) Immunofluorescent micrograph showing P-IV in the mesangium (M) and, in continuity, along the peripheral capillary (small arrows). (B) Phase-contrast micrograph of the same section demonstrating the dark line of the lamina densa (large arrow) and the mesangium (M). Note the "halo" effect of phase microscopy along the epithelial aspect of the lamina densa and to a lesser extent along its internal aspect. (C) Combination immunofluorescent and phase-contrast microscopy of the section shown in A and B. Note that positive immunofluorescence for P-IV is seen in the mesangium (M) and along the internal aspect (small arrow) of the lamina densa (large arrow). The nonfluorescent phase "halo" is visible along the epithelial part of the lamina densa as seen in B, whereas the white line along the internal aspect of the GBM is specific immunofluorescence as in A, and not a "halo" effect (×900).

not in the mesangium. Laminin was present primarily in a DL pattern on both sides of the phase-dense portion of the GBM as visualized by phase-contrast microscopy and to a lesser degree within the mesangium. The GBM of intact tissue in sonicated or detergent-extracted material also stained positively (1–2+) for albumin.

Although the normal anatomy of the glomerulus was generally disrupted after sonication, there was an adequate number of areas in which the normal relationships of the mesangium and GBM could be identified. Sonicated material contained all of the antigens previously noted in a distribution similar to that present in detergent-extracted material. However, it was not possible to quantitate the amount of mesangial material in sonicated preparations by morphometric analysis or by immunofluorescence microscopy. In GBM isolated by either technique, a relative increase in staining intensity in the GBM was observed with all reagents.

Immunofluorescence similar to that seen in human tissue was observed when sections of intact rat kidney or detergent-extracted material were stained with antisera to P-IV, $\alpha A \alpha B$, fibronectin, actomyosin, and GP antigen. In contrast to the human, SL staining was observed with anti-laminin and anti-BLC-IV stained the mesangium more intensely than it stained the GBM.

DISCUSSION

Isolated GBM is a heterogeneous structure containing site-specific arrays of different antigens that bear the same relationship to the lamina densa as that seen in thin tissue sections using phase-contrast immunofluorescence microscopy. Indeed, an array of antigens that includes P-IV, $\alpha A \alpha B$, and fibronectin appear to be along the internal aspect of the phase-dense image of peripheral lamina densa and contiguous with the same antigens in the mesangial matrix. In contrast, the linear immunofluorescence observed with antisera to BLC-IV superimposes the phase-dense GBM. The reason that antibody to P-IV and antibody to BLC-IV react with distinctly different loci within the glomerulus is unknown. It is likely that the antigenic determinants are present in different sites, although it is also possible that the noncollagen domain of P-IV reacting with the antibody is unexposed in the GBM. GP antigen and amyloid P component are also seen in a SL pattern along the GBM but not in the mesangium, whereas laminin is present on both sides of the lamina densa in a DL pattern and in the mesangium. Even actomyosin persists in the mesangium of isolated GBM, demonstrating that the mesangial zone contains components derived from the mesangial cell in addition to matrix material.

The sharing of certain antigens by the peripheral

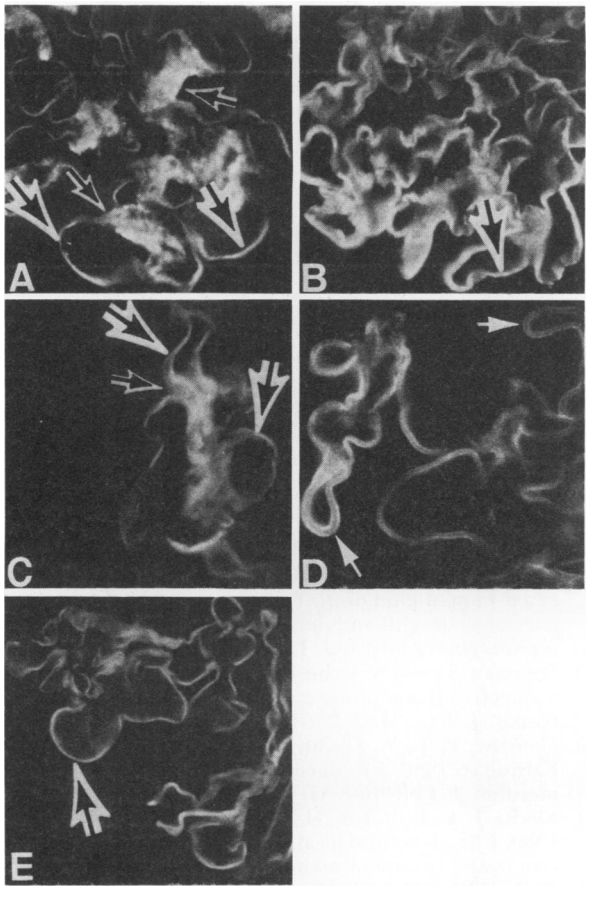

FIGURE 4 Immunohistochemical analysis of human GBM isolated by detergent extraction. Tissues stained with antisera reactive to: (A) type V collagen (×600); (B) IV-BLC collagen (×500); (C) fibronectin (×700); (D) laminin (×700); (E) GP (×500). Small arrow denotes mesangial matrix, large arrow denotes staining along the peripheral GBM in a linear pattern, white arrow denotes double linear staining of the peripheral GBM.

GBM and the mesangium suggests a similarity between these two sites although there is no information regarding the relative amounts that are present. However, there are clear differences without overlap as indicated by the distribution of the GP antigen exclusively within the GBM. The demonstration of type V collagen by immunohistochemical techniques illustrates the usefulness of this approach for detecting antigens that are present in small amounts and not identified by biochemical techniques.

The effects of more intense sonication on antigen loss could not be evaluated, although prolonged sonication has been reported to remove glycosaminoglycans (3), whereas albumin bound to normal and diabetic GBM persists as demonstrated by radioimmunoassay (30). Indeed, in the present study albumin was detected in the GBM of intact tissue as previously reported (26) but was also demonstrated in detergent-extracted and sonicated GBM, confirming prior results obtained by radioimmunoassay on collagenase-digested GBM (30). The mechanism of binding of albumin to normal GBM or, in significantly higher amounts, to diabetic GBM is unknown. Nevertheless, these findings support the concept that certain antigens in GBM (e.g. fibronectin and amyloid P component) might be derived from plasma although there is no information at present to prove or disprove this hypothesis. However, it is unlikely that this mechanism plays a role in the localization of collagen and glycoprotein antigens described in this paper.

In addition, our immunohistochemical and morphometric studies demonstrate that a large proportion of the material present in detergent-isolated GBM is mesangial material. Although previously recognized by transmission electron microscopy (31), the current studies demonstrate that detergent-isolated GBM contains mesangial material in an amount at least equivalent to that present in intact glomeruli. The higher percentage of matrix in isolated glomeruli and GBM compared with that in intact tissue may represent a sampling defect or may be a consequence of the isolation procedure. An accurate assessment of the total GBM thickness after either isolation procedure in the rat was not possible because lamina rarae cannot be visualized by standard electron microscopy. The apparent lamina densa in these preparations was significantly increased in thickness, however. This may reflect swelling in detergent-treated GBM; in sonicated preparations "fraying" of the basement membrane has been noted previously (19).

Our morphometric and immunohistochemical data do not permit an assessment of the absolute amount of the various antigens present within the GBM nor the relative change in components induced by the isolation procedure. In addition, the exact relationship of these antigens to the lamina densa and the lamina rara interna and externa must await further analysis and the refinement of immunoelectron microscopic techniques. The results of this investigation bear directly on the interpretation of studies of isolated GBM—a heterogeneous structure. For example, attempts to define changes in diabetic GBM on the basis of compositional studies alone have led to conflicting results (32–34)—a situation perhaps not unexpected in view of the number of antigenic components now recognized in isolated material as well as the fibrotic nature of the kidneys used in these studies. An alteration induced by disease in one antigenic component of a heterogeneous structure may not be recognized by

standard biochemical analyses of the whole structure, especially if the particular component is present in relatively low concentrations. In this context, the demonstration of heparan sulfate in GBM by morphologic techniques (3) preceded its recognition and isolation by biochemical analysis.

At present, the relationship of these antigens, recognized by immunofluorescence, to biochemically-isolated components from GBM is largely unexplored. More importantly, the occurrence of site-specific arrays of antigens provide the foundation for future studies regarding the role of individual components in the regulation of macromolecular transport and in specific disease states.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Crystal Blocher and Lore Lang for technical assistance, Mr. Marshall Hoff for photographic assistance, and Ms. Jan Aplin for secretarial assistance.

This work was aided by grants from the National Institutes of Health (AI10704, AM26149, AM21956, and AM25518) and the Minnesota Vikings Children's Fund American Heart Association, American Diabetes Association. Dr. Houser was a postdoctoral fellow supported by the National Institutes of Health (AM07087).

REFERENCES

1. Kefalides, N. A. 1971. Isolation of a collagen from basement membranes containing three identical α-chains. *Biochem. Biophys. Res. Commun.* **45:** 226–234.
2. Sato, T., and R. G. Spiro. 1976. Studies on the subunit composition of the renal glomerular basement membrane. *J. Biol. Chem.* **251:** 4062–4070.
3. Kanwar, Y. S., and M. G. Farquhar. 1979. Presence of heparan sulfate in the basement membrane. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 1303–1307.
4. Cohen, M. P., and M. Surma. 1980. Renal glomerular basement membrane. In vivo biosynthesis and turnover in normal rats. *J. Biol. Chem.* **255:** 1767–1770.
5. Meezan, E., J. T. Hjelle, and K. Brendel. 1976. A simple, versatile, nondisruptive method for the isolation of morphologically and chemically pure basement membranes from several tissues. *Life Sci.* **17:** 1721–1732.
6. West, T. W., J. W. Fox, M. Jodlowski, J. W. Freytag, and B. G. Hudson. 1980. Bovine glomerular basement membrane. Properties of the collagenous domain. *J. Biol. Chem.* **255:** 10451–10459.
7. Crouch, E., H. Sage, and P. Bornstein. 1980. Structural basis for apparent heterogeneity of collagens in human basement membranes: type IV procollagen contains two distinct chains. *Proc. Natl. Acad. Sci. U. S. A.* **77:** 745–749.
8. Scheinman, J. I., J-M. Foidart, and A. F. Michael. 1980. The immunohistology of glomerular antigens. V. The collagenous antigens of the glomerulus. *Lab. Invest.* **43:** 373–381.
9. Scheinman, J. I., A. J. Fish, and A. F. Michael. 1974. The immunohistopathology of glomerular antigens. The glomerular basement membrane, collagen, and actomyosin antigens in normal and diseased kidneys. *J. Clin. Invest.* **54:** 1144–1154.
10. Scheinman, J. I., A. J. Fish, A. J. Matas, and A. F. Michael. 1978. The immunohistopathology of glomerular antigens. II. The glomerular basement membrane, actomyosin, and fibroblast surface antigens in normal, diseased, and transplanted human kidneys. *Am. J. Pathol.* **90:** 71–84.
11. Scheinman, J. I., J-M. Foidart, P. Gehron-Robey, A. J. Fish, and A. F. Michael. 1980. The immunohistology of glomerular antigens. IV. Laminin, a defined noncollagen basement membrane glycoprotein. *Clin. Immunol. Immunopathol.* **15:** 175–189.
12. Fish, A. J., K. M. Carmody, and A. F. Michael. 1979. Spatial orientation and distribution of antigens within human glomerular basement membrane. *J. Lab. Clin. Med.* **94:** 447–457.
13. Roll, F. J., J. A. Madri, J. Albert, and H. Furthmayr. 1980. Codistribution of collagen types IV and AB_2 in basement membranes and mesangium of the kidney. *J. Cell Biol.* **85:** 597–616.
14. Pettersson, E. E., and R. B. Colvin. 1978. Cold-insoluble globulin (fibronectin, LETS Protein) in normal and diseased human glomeruli: Papain-sensitive attachment to normal glomeruli and deposition in crescents. *Clin. Immunol. Immunopathol.* **11:** 425–436.
15. Stenman, S., and A. Vaheri. 1978. Distribution of a major connective tissue protein, fibronectin, in normal human tissues. *J. Exp. Med.* **78:** 1054–1064.
16. Courtoy, P. J., Y. S. Kanwar, R. O. Hynes, and M. G. Farquhar. 1980. Fibronectin localization in the rat glomerulus. *J. Cell Biol.* **87:** 691–696.
17. Madri, J. A., R. J. Roll, H. Furthmayr, and J-M. Foidart. 1980. Ultrastructural localization of fibronectin and laminin in the basement membranes of the murine kidney. *J. Cell Biol.* **86:** 682–687.
18. Dyck, R. F., C. M. Lockwood, M. Kershaw, N. McHugh, V. C. Duance, M. L. Baltz, and M. B. Pepys. 1980. Amyloid P component is a constituent of normal human glomerular basement membrane. *J. Exp. Med.* **152:** 1162–1174.
19. Westberg, N. G., and A. F. Michael. 1970. Human glomerular basement membrane. Preparation and composition. *Biochem. J.* **9:** 3837–3846.
20. Blau, E., and A. F. Michael. 1971. Rat glomerular basement membrane composition and metabolism in aminonucleoside nephrosis. *J. Lab. Clin. Med.* **77:** 97–109.
21. Rennard, S. I., R. Berg, G. R. Martin, J-M. Foidart, and P. Gehron-Robey. 1980. Enzyme-linked immunoassay (ELISA) for connective tissue components. *Anal. Biochem.* **104:** 205–214.
22. Gunson, D. E., and N. A. Kefalides. 1976. The use of the radioimmunoassay in the characterization of antibodies to basement membrane collagen. *Immunology.* **31:** 563.
23. Skinner, M., J. L. Vaitukaitis, A. S. Cohen, and M. D. Benson. 1979. Serum amyloid P component levels in amyloidosis, connective tissue diseases, infection, and malignancy as compared to normal serum. *J. Lab. Clin. Med.* **94:** 633–638.
24. Benson, M. D., and M. Skinner. 1978. Amino terminal sequence of amyloid P component isolated from serum. *J. Lab. Clin. Med.* **92:** 78–83.
25. Scheinman, J. I., M. Steffes, D. Brown, and M. Mauer.

1978. The immunohistopathology of glomerular antigens. III. Increased actomyosin in experimental diabetes in the rat. *Diabetes.* **27:** 632.
26. Miller, K., and A. F. Michael. 1976. Immunopathology of renal extracellular membranes in diabetes mellitus: Specificity of tubular basement membrane fluorescence. *Diabetes.* **25:** 701–708.
27. Steffes, M. W., D. M. Brown, J. M. Basgen, A. J. Matas, and S. M. Mauer. 1979. Glomerular basement membrane thickness following islet transplantation in the diabetic rat. *Lab. Invest.* **41:** 116–118.
28. Jensen, E. B., H. J. G. Gundersen, and R. Osterby. 1979. Determination of membrane thickness distribution from orthogonal intercepts. *J. Microsc. (Oxf.).* **116:** 19–33.
29. Steffes, M. W., D. M. Brown, J. M. Basgen, and S. M. Mauer. 1980. Amelioration of mesangial volume and surface alterations following islet transplantation in diabetic rats. *Diabetes.* **29:** 509–515.
30. Michael, A. F., and D. M. Brown. 1981. Increased concentration of albumin in kidney basement membranes in diabetes mellitus. *Diabetes.* **30:** 843–846.
31. Meezan, E., K. Bendel, J. T. Hjelle, and E. C. Carlson. 1978. A versatile method for the isolation of ultrastructurally and chemically pure basement membrane with sonication. *In* Biology and Chemistry of Basement Membranes. Edited by N. Kefalides. Academic Press, Inc., New York.
32. Beisswenger, P. J., and R. G. Spiro. 1970. Human glomerular basement membrane: Chemical alteration in diabetes mellitus. *Science (Wash. D. C.)* **168:** 598.
33. Westberg, N. G., and A. F. Michael. 1973. Human glomerular basement membrane: Chemical composition in diabetes mellitus. *Acta Med. Scand.* **194:** 39–47.
34. Kefalides, N. 1974. Biochemical properties of human glomerular basement membranes in normal and diabetic kidneys. *J. Clin. Invest.* **53:** 403–407.