Kidney International, Vol. 47 (1995), pp. 1078–1086

Direct podocyte damage in the single nephron leads to albuminuria *in vivo*

WIM LAURENS, CRISTINA BATTAGLIA, CHIARA FOGLIENI, RITA DE VOS, BARBARA MALANCHINI, BOUDEWIJN VAN DAMME, YVES VANRENTERGHEM, GIUSEPPE REMUZZI, and ANDREA REMUZZI

Mario Negri Institute for Pharmacological Research and Division of Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Bergamo, Italy, and Departments of Nephrology and Pathology, Catholic University of Leuven, Leuven, Belgium

Direct podocyte damage in the single nephron leads to albuminuria *in vivo*. The hypothesis that detachment of podocytes leads to albuminuria was tested by studying the single nephron albuminuria *in vivo* after injecting a saponin solution (0.6 mg/ml) in Bowman's space of superficial glomeruli, which produces selective damage of the podocytes, in female Munich-Wistar-Frömter rats. Animals were subsequently installed under a fluorescence microscope, a purified fluoresceinated rat albumin solution was intravenously injected and the passage of the fluorescent albumin was followed through the microscope. Of the 47 glomeruli injected with the saponin solution (in 6 animals) 46 became fluorescent within seconds with the fluorescence progressing from Bowman's space into the proximal tubule and then in the rest of the tubule. In superficial non-injected and control-injected glomeruli weak fluorescence could be detected only in the glomerular tuft and the peritubular capillaries. Tubuli injected with the saponin solution remained indistinguishable from non-injected tubuli. Electron microscopic study of the saponin-injected glomeruli confirmed the selective removal of podocytes. Immunogold electron microscopy confirmed that the intact albumin molecule effectively passed the glomerular capillary wall at the site where podocyte detachment had occurred. It is concluded that selective removal of podocytes at the single nephron level leads to albuminuria *in vivo*, and therefore podocytes play a crucial role in regulating the permeability of the glomerular capillary wall.

Several experimental observations suggest that podocytes play an important role in determining the permselective function of the glomerular capillary wall (GCW). It has already been shown previously that ferritin, a macromolecular tracer, crosses the capillary wall at the site of podocyte detachment [1] and that isolated basement membrane has a much higher permeability for dextrans than intact glomeruli *in vitro* [2]. Also, clinical conditions such as minimal change nephropathy where extensive fusion of the foot processes at the electronmicroscopical level is the only pathological finding are accompanied by proteinuria [3]. However up till now there was no direct experimental proof that selective podocyte damage leads to an increased glomerular filtration of plasma proteins *in vivo*.

Recently we demonstrated that injection of a saponin (S)-solution in Bowman's space of superficial glomeruli with a micropuncture technique induces direct damage to the podocytes, without ultrastructural lesions to the endothelial cells or to the basement membrane, and leads to the development of focal and segmental glomerulosclerosis (FSG) [4]. In the present study we investigated whether segmental removal of the podocytes induced by this procedure leads to the loss of the permselective properties of the glomerular capillary wall (GCW) *in vivo*. To this purpose we selectively damaged podocytes in surface glomeruli of female Munich-Wistar-Frömter (MWF)/Ztm rats and evaluated single nephron albuminuria in the intact living kidney. We used *in vivo* fluorescence microscopy to detect glomerular filtration of intravenously injected fluorescent rat albumin in S-injected and in glomeruli injected with a saponin-free solution for comparison. Biopsies of the S-injected and control glomeruli were taken after the fluorescence study for transmission electron microscopy. Additional saponin-injected and control glomeruli were studied by immunogold electronmicroscopy.

Received for publication April 4, 1994
and in revised form November 14, 1994
Accepted for publication November 14, 1994

Methods

Experimental design

For these experiments six female Munich-Wistar-Frömter (MWF)/Ztm rats were used. In contrast to the males, females of this strain do not develop spontaneous progressive proteinuria [5] or focal and segmental glomerulosclerosis [6]. The weight of the animals was between 160 and 220 g and the proteinuria was below 30 mg/24 hr.

Micropunctures were performed as described previously, with slight modifications [4, 7]. Briefly, animals were anesthetized with Inactin (Byk Gulden Konstanz, Germany) 100 mg/kg intraperitoneally, placed on a constant temperature controlled micropuncture table and tracheostomized. The left femoral artery was catheterized for continuous arterial pressure recording (Battaglia, Rangoni, Bologna, Italy). A polyethylene catheter was inserted for infusion of saline 1 ml/hr and fluoresceinated rat albumin. Through a left ventral incision the left kidney was gently freed from the surrounding fat, placed in a Lucite holder, immobilized by partial embedding in Agar 3% (Agarose SERVA high EEO, Heidelberg, Germany) and constantly irrigated with saline at 37°C. Micropipettes with a classic beveled tip and an external diameter of 10 μm were filled with the saponin (S) solution (Sigma Chemical Company, St. Louis, MO, USA) and mounted on a microinfusion pump (Microperfusionspumpe 1 to 50 nl/min; Wolfgang Hampel, Neu-Isenburg, Germany) allowing a constant

delivery of pre-set minute amounts of fluid. Micropuncture of Bowman's space of superficial glomeruli was performed to infuse 10 nl of a 0.6 mg/ml S-solution in 0.05% Lissamine green in saline at a rate of 10 nl/min. Other superficial glomeruli were injected in the same way using a control (C) solution of 0.05% Lissamine green in saline. First we injected about eight superficial glomeruli with the C-solution, whereafter the micropipette of the microinfusion pump was exchanged with a micropipette containing the S-solution. Then seven to nine superficial glomeruli were injected with the S-solution, depending upon the anatomical availability of superficial glomeruli. The microinjections were performed in such a way that in the same microscopic field one or two glomeruli injected with the S-solution, one or two glomeruli injected with the C-solution and a few non-injected superficial glomeruli could be observed. In total also 10 tubuli were injected with the S-solution. Finally a drawing was made of the injected structures to allow easy identification under the fluorescence microscope.

Fluorescent labeling of rat albumin

Rat albumin was labeled with 5,(6)-carboxyfluorescein N-hydroxysuccinimide ester (F) (Boehringer-Mannheim) according to the instructions of the manufacturer. Globulin-free rat albumin (Sigma Chemical Company) was dissolved at 25 mg/ml in 0.1 M carbonate buffer at pH 8.5. The fluorescent dye was prepared in DMSO and mixed rapidly with the rat albumin solution at a final dye-to-protein molar ratio of 10:1. After about two hours incubation at room temperature on gentle shaking, the conjugate was separated from unbound dye by Sephadex G-50 chromatography in 0.1 M carbonate buffer. The fractions containing F-rat albumin were pooled and then dialyzed in the dark at 4°C for 48 hours against two changes of 0.9 % sodium chloride. After dialysis, the molar F-to-albumin ratio of the final product was determined by measuring the adsorption at 280 nm and at 496 nm. The concentration of F-albumin was estimated by the method of Read and Northcote [8]. The purity of the fluorescence albumin was analized by SDS-PAGE according to Laemnli [9]. The conjugate was kept at 4°C in the dark and used within one week.

In vivo fluorescence microscopy

Immediately after the micropuncture the rat was installed on a special self-devised table mounted on the microscope stage of a fluorescence microscope (Olympus BH2-RFCA, Tokyo, Japan) equipped with a photocamera (Olympus OM-4) and a video camera (JVC TK-S200, Tokyo, Japan) connected to a television monitor (JVC TM-920). The kidney was placed in a Lucite holder mounted on the table, partially embedded in Agar 3% and irrigated with saline at 37°C. Blood pressure was continuously monitored through the arterial catheter and 40 minutes after the first microinjection with the S-solution, 20 mg/kg body weight of the purified F-rat albumin was injected in the left femoral vein. The appearance of the fluorescence in the different anatomical structures was monitored through the microscope and documented with photographs.

Transmission electron microscopy

Biopsies of some S-injected glomeruli were taken four hours after the microinjections, after finishing the observations under the fluorescence microscope, in the *in situ* kidney with a self-devised miniature biopsy needle as previously described [4]. The biopsies, kept in the dark during the processing, were first fixed in glutaraldehyde for four hours, and then the fluorescent dye was photoconverted by treatment with diaminobenzidine in order to transform the injected F-albumin in an electron-opaque oxidation product [10–12]. Processing was carried out routinely through progressive dehydration steps and biopsies were embedded in epon. During the embedding the kidney surface side of the biopsy was oriented towards the sectioning side of the tissue block and the injected glomerulus was localized in semithin sections of 0.8 μm thickness. Ultrathin sections of 0.5 mm thickness were only stained with uranyl acetate to detect the photoconverted fluorescent albumin and were subsequently analyzed with a transmission electron microscope (EM 109, Zeiss). Several serial ultrathin sections of the injected and normal glomeruli were studied.

Immunogold electronmicroscopy

For immunoelectronmicroscopy a small kidney sample containing the saponin treated glomerulus as well as a control kidney sample was fixed according to a previously described method [13]. Samples were infiltrated and polymerized in LR white resin according to a method modified after Timms [14], Fernando et al [15], and Scala et al [16] (De Vos, unpublished results). Thereafter a post-embedding immunogold staining method was applied by incubating the tissue sections overnight at 4°C with the specific IgG fraction of a polyclonal rabbit antibody to rat albumin (CAPPEL Research Products, catalog no. 55728, Organon Teknika Corporation, USA) in a dilution of 1/200. Subsequently sections were incubated with the immunogold probe GAR IgG 20 nm (Biocell, UK) and counterstained with uranyl acetate and lead citrate. Controls were processed by omitting the primary antibody and/or by incubation with pre-immune serum.

Results

Characterization of rat albumin labeling

SDS-PAGE of F-rat albumin samples after dialysis revealed a single band with a molecular weight of approximately 64,000 Daltons and the electrophoretic behavior of the F-rat albumin was indistinguishable from unlabeled rat albumin. No free F was visible at or ahead of the dye front of the gel under UV illumination, and the albumin band was both fluorescent and Coomassie Blue stained. The spectrophotometrically determined molar dye/protein ratio of albumin was ~1.4. The final concentration of F-rat albumin was 2.5 mg/ml.

In vivo fluorescence microscopy

In total 47 glomeruli and 10 tubuli were injected with the S-solution and 46 glomeruli with the C-solution. Before the injection of the F-rat albumin neither in the saponin, nor in control injected glomeruli spontaneous fluorescence could be observed. As shown in Figure 1, after F-albumin injection weak fluorescence in non-injected glomeruli was only apparent in the glomerular tuft and in the peritubular capillaries (and occasional bigger vessels apparent on the kidney surface). In 46 of the 47 S-injected glomeruli fluorescent staining appeared within seconds not only in the glomerular tuft, but also in Bowman's space, the proximal tubule and then progressively in the rest of the tubule. In several S-injected glomeruli fluorescent ultrafiltrate could be noted leaking out of Bowman's capsule at the site of unsealed holes of the micropuncture. As shown in Figure 1 from the 46 C-injected glomeruli only the glomerular tuft became weakly

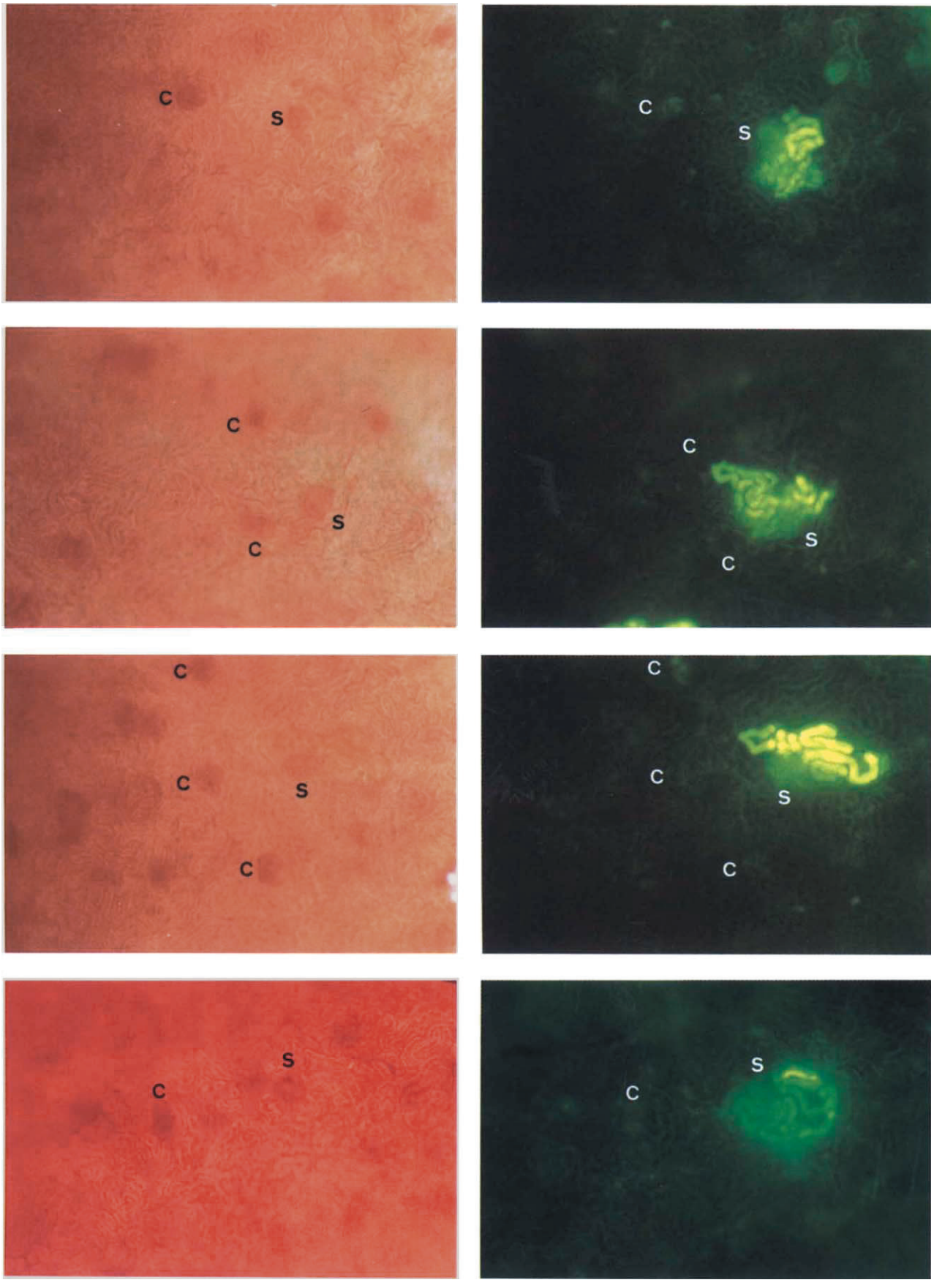
C
S
C
S
C
S
C
C
S
C
C
S
C
C
S
C
C
S
C
S
C
S

Fig. 1. *Photographs of surface glomeruli injected with saponin (S)-solution (0.6 mg/ml) or control (C)-solution as seen with normal light are shown on the left, unlabeled glomeruli are non-injected.* On the right the same microscopic fields as seen with fluorescent light are shown: only the tubules of S-injected glomeruli are fluorescent after the intravenous injection of F-rat albumin. Tubules of control and non-injected glomeruli are not fluorescent. Reproduction of this figure in color was made possible by a grant from Zeneca, Destelbergen–Belgium.

fluorescent, and these glomeruli were in terms of fluorescence indistinguishable from the non-injected glomeruli. This observation demonstrates that the increased albumin filtration in S-injected glomeruli is due to the lysis of the podocytes by the S-solution and not to mechanical damage of the glomerular capillary wall induced by the tip of the pipette. The S-injected tubuli showed no intraluminal fluorescence and were indistinguishable from non-injected tubuli.

Transmission electron microscopy

As shown in Figure 2, the electron microscopic findings four hours after the injection of the S-solution are comparable with the results we described previously after 40 minutes. In detail, in the glomeruli injected with the S-solution segmental lysis of the podocytes was observed and cellular debris was present in the urinary space. Some podocyte bodies were detached from the GBM and their foot processes disappeared or were detached without identifiable slit diaphragms. Others showed extensive fusion of the foot processes. The basement membrane, the endothelial and the mesangial cells were normal and indistinguishable from the corresponding structures in the non-injected glomeruli. As previously reported, in some denuded capillary lumina there was an accumulation of red blood cells surrounded by plasma. A segmental lysis of parietal epithelial cells was also observed. The photoconversion product of the F-albumin was mainly present in capillary lumina and could not be demonstrated in the urinary space in normal glomeruli. On the contrary, in the S-injected glomeruli, the photoconversion product of the F-albumin was identified both in the capillary lumina and in the urinary space (Fig. 2).

Immunogold electronmicroscopy

In the capillaries of a control glomerulus the gold particles were retained at the endothelial side of the basement membrane and no gold particles could be detected in Bowman's space (Fig. 3).

In a saponin treated glomerulus gold particles permeated the basement membrane at the site of the podocyte detachment and multiple gold particles could be noted in Bowman's space. There was also an apparent denser labeling with the immunogold intracapillary. In this oblique section of the capillary segment, the intact fenestrated endothelium could be seen very clearly (Fig. 4).

Discussion

Our present study is the first *in vivo* demonstration that direct damage to the podocytes leads rapidly to albuminuria. As shown previously, microinjection of 10 nl of a 0.6 mg/ml saponin solution in Bowman's space induces segmental lysis of the podocytes without ultrastructural damage to the basement membrane and the endothelial or mesangial cells, as observed in biopsies taken 40 minutes after the microinjection [4]. In the present study fluorescent rat albumin was injected intravenously and evaluated its appearance in the proximal tubules of surface glomeruli 40 minutes after the first saponin microinjection. In the saponin-injected glomeruli fast staining of the tuft, Bowman's space and the proximal tubule was seen after the F-albumin was injected. In non-injected glomeruli and in glomeruli injected with the control solution only weak fluorescence appeared in the glomerular tuft and in the peritubular capillaries, whereas no staining could be observed in the proximal and distal tubules. These observations indicate that the purified F-albumin was effectively restricted to the lumen of glomerular capillaries with undetectable filtration into the urinary space, while in saponin-injected glomeruli an important increase in glomerular filtration of circulating albumin took place. Electronmicroscopy observations confirm that in the saponin-injected glomeruli the photoconversion product of the F-rat albumin was present both in the capillary lumen and the urinary space, whereas in normal glomeruli it was mainly present in the capillary lumen and almost negligible in the urinary space. Moreover, the immunogold electronmicroscopy experiments, where as primary antibody a specific IgG fraction of a polyclonal rabbit anti-rat albumin antibody was used, demonstrate that the intact albumin molecule permeates the basement membrane at the site of the podocyte detachment.

It is well known that the GCW almost completely restricts the passage of large plasma proteins, such as albumin, and at the same time allows a high filtration rate of plasma water and small solutes. Plasma proteins are retained in the capillary lumen as the result of charge and size selective function of the GCW. The precise mechanisms of the filtration barrier to proteins, however, are not yet completely understood. Of the three components of the GCW (endothelial cells, basement membrane and podocytes), endothelial cells, due to their large open fenestrae, have not been implicated in forming a major resistance to plasma proteins or water [17], while the basement membrane and the podocytes are believed to be the morphological and functional barrier to macromolecule filtration. Recent immunohistochemical studies have demonstrated that the GBM acts both as an effective size and charge selective barrier for endogenous proteins [18, 19]. However, other groups have demonstrated that high molecular weight dextran and ferritin penetrate the glomerular basement membranes of the acellular perfused rabbit kidney [20], that exogenous tracers, like ferritin, permeate the GCW at sites of podocyte detachment [1], that podocyte detachment correlates with proteinuria in adriamycin and puromycin nephrosis [21], and that isolated GBM *in vitro* has a much higher permeability for large dextrans than intact isolated glomeruli [2].

As shown previously [4], the ultrastructural lesions we observed in the saponin-injected glomeruli, from biopsies taken after the fluorescence study, are limited to the epithelial cells and no structural damage to the GBM, the endothelial or the mesangial cells was noted. That in our experimental condition basement membrane maintains its structural integrity after contact with detergent solutions is suggested by several other investigations. In previous studies saponin concentrations up to 1 mg/ml and contact times up to 45 minutes were used to remove endothelial cells from arterial walls leaving the underlying basement membrane intact [22–24]. Another detergent, sodium deoxycholate 4%, with a contact time of two hours, was used to isolate

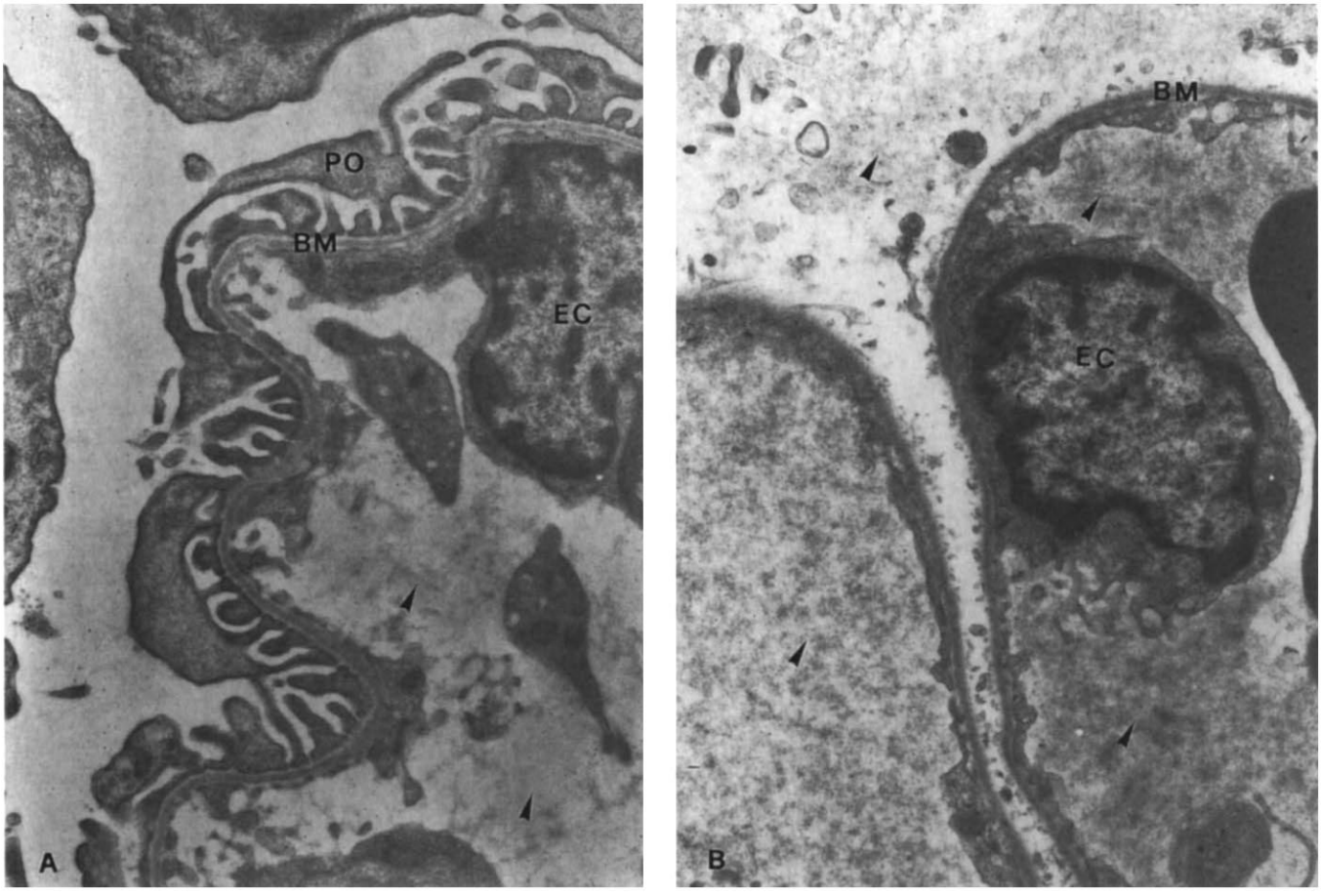

Fig. 2. *Electronmicrographs (final magnification ×12,000) of a capillary of a normal glomerulus (A) and of a capillary of a S-injected glomerulus (B).* (A) In the normal capillary the photoconversion product of the F-rat albumin (arrows) is confined to the capillary lumen. Podocytes (PO), basement membrane (BM) and endothelial cells (EC) are normal. (B) In the capillary of the S-injected glomerulus podocytes (PO) are selectively lysed. Basement membrane (BM) and endothelial cells (EC) have the same ultrastructural appearance as in the normal capillary. The photoconversion product of the F-rat albumin (arrows) is present in both the capillary lumen and the urinary space.

basement membranes with preservation of its ultrastructural appearance and immunohistochemical defined antigens [25, 26]. In the acellular perfused rabbit kidney model sequential perfusions of the isolated kidney with the cell detergents Triton X-100 0.5%, Triton X-100 3% and sodium deoxycholate 4%, each time for several hours, finally yielded an acellular kidney with ultrastructurally intact basement membranes as demonstrated by transmission electronmicroscopy [20]. Also, recent permeability studies of the glomerular basement membrane used a detergent (0.5% N-laurylsarcosine) with a contact time of 45 minutes to isolate basement membrane with an apparently intact structure and composition [2]. Finally, the present study and our previous report [4] show that the ultrastructure of basement membrane of capillaries denuded of podocytes is not different from basement membrane in normal capillaries.

All these studies, however, do not exclude the possibility that detachment of podocytes induces conformational changes of the basement membrane structure at the molecular level. That the integrity of podocyte attachment to the GBM plays a role in regulating the permeability is suggested by the fact that selective removal of podocytes leads to an increased permeability for albumin, as shown here *in vivo*, and that in the intact GCW albumin is mainly restricted at the lamina rara interna of the basement membrane as we have shown by ultrastructural localization of the endogenous rat albumin with an immunogold staining technique, confirming previous reports [19]. That podocytes might play an important role in the pathogenesis of proteinuria is also based on observations that it is possible to induce immediate proteinuria with the F(ab')2 fragment of anti-Fx1A [27], with antibodies directed to different glycoproteins present on podocytes [28–30], with noncomplement-fixing-γ2-subclass IgG of sheep anti-rat nephrotoxic serum directed against a β1-integrin on podocytes [31], and with a monoclonal antibody against a mouse aminopeptidase A, an angiotensinase, present on podocytes [32]. Recent evidence suggests that $\alpha 3\beta 1$-integrin plays an important role in this attachment of the podocytes to basement membrane proteins [33]. This integrin is linked through talin and vinculin to the actin-cytoskeleton of the podocytes [34]. It has been reported that angiotensin II leads to an increased aggregation of actin in podocytes with widening of the foot processes [35, 36] and atrial natriuretic peptide leads to actin disaggregation with smaller foot processes [36]. It is tempting to speculate that through these mechanisms the podocytes might regulate the permeability to macromolecules of the GCW with their contractile cytoskeleton.

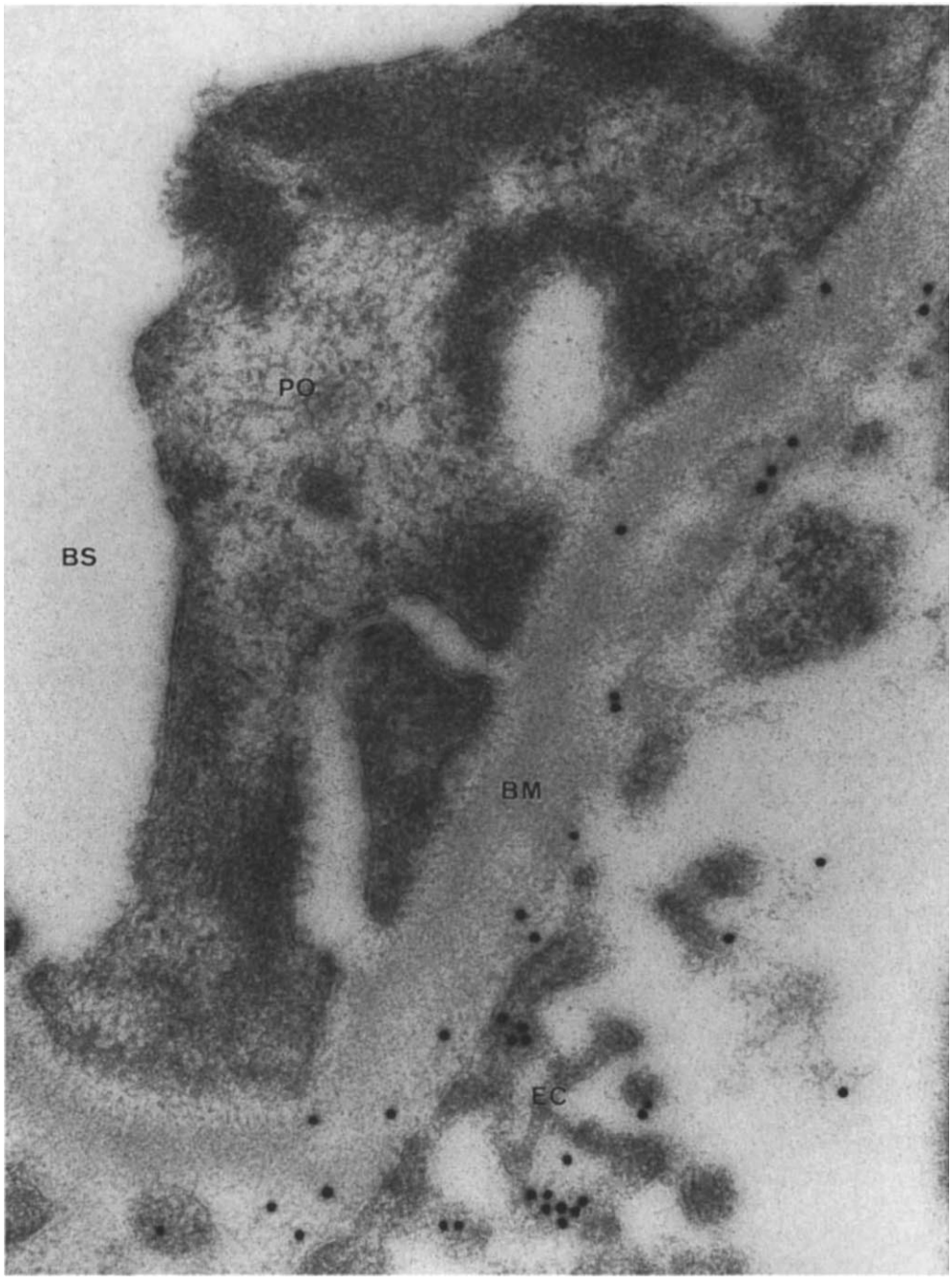

Fig. 3. *Immunogold electronmicrographs (final magnification ×31,500) of capillary of normal glomerulus.* The endogenous rat albumin was labeled by applying the specific IgG fraction of a polyclonal rabbit anti-rat albumin antibody overnight at 4°C on a LR white embedded tissue section. In a second step, labeling was completed using the immunogold probe GAR IgG 20 nm. The gold labeled albumin is retained at the endothelial side of the intact glomerular capillary wall. No gold particles can be seen in Bowman's space (BS). Podocytes (PO), basement membrane (BM) and endothelial cells (EC) are normal.

The findings that a monoclonal antibody against a mouse aminopeptidase A, an angiotensinase, present on podocytes leads to an immediate albuminuria with widening and "fusion" of the foot processes [32] and that ACE inhibitors and angiotensin II receptor antagonists improve the permselectivity of the GCW [37, 38] are strong arguments in favor of such a mechanism. Moreover, the hypothesis that podocytes regulate the permeability of the GCW is in line with observations showing that the GBM is not a rigid but a distensible structure and that podocytes might counteract GCW distension through their contractile cytoskeleton attached at the GBM [39].

Our present observation that podocyte damage induces abnormal albumin filtration would suggest that there might be a link between this early dysfunction of the glomerular membrane and

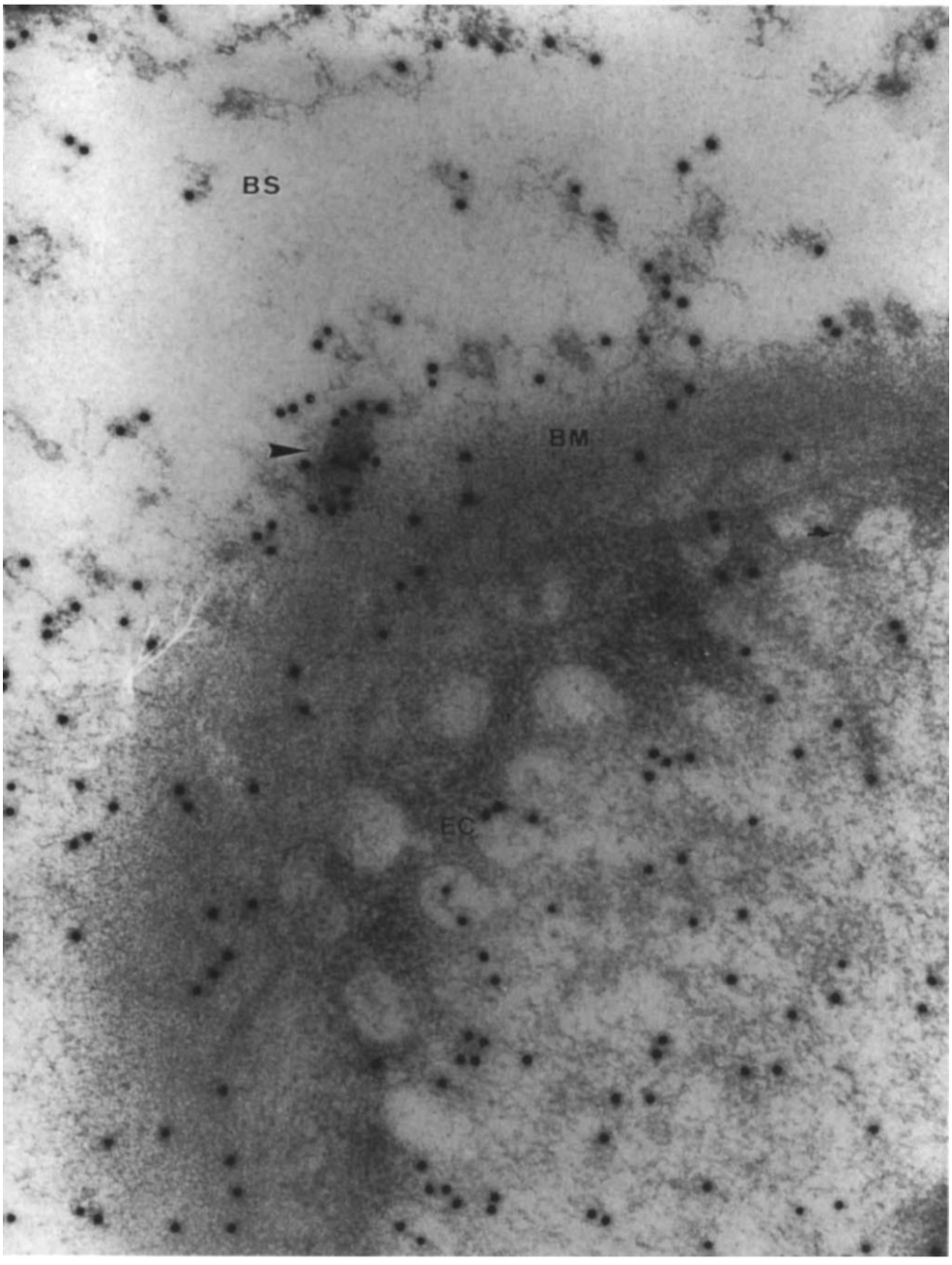

Fig. 4. *Immunogold electron micrographs (final magnification ×31,500) of an oblique section of a capillary of a S-injected glomerulus.* The endogenous rat albumin was labeled by applying a specific IgG fraction of a polyclonal rabbit anti-rat albumin antibody overnight at 4°C on a LR white embedded tissue section. In a second step, labeling was completed using the immunogold probe GAR IgG 20 nm. This oblique section of the capillary very clearly shows the completely intact fenestrated endothelial cells (EC). Podocytes are removed from this capillary segment apart from a small remnant (arrow). The gold labeled albumin is permeating the basement membrane denuded of podocytes. Many gold labeled albumin particles can be seen in Bowman's space (BS). There is also an apparent concentration of gold labeled albumin particles in the lumen of the capillary. Basement membrane (BM) and endothelial cells (EC) are normal.

subsequent development of glomerulosclerosis. However, the precise mechanisms linking these two phenomena cannot be established on the basis of the present investigation. In addition to the increased albumin filtration one can speculate that water filtration could also be increased in saponin treated glomeruli. As podocytes are responsible for more than half of the hydraulic resistance of the GCW [17], the filtration of water and small solutes will be much higher in capillaries without podocytes. On the basis of these considerations, local glomerular hemodynamic conditions could be strongly affected, and this is suggested by the

apparent hemoconcentration in these capillaries that has been observed in this and in the previous report, and by the concentration of immunogold labeled albumin in the present immunogold electronmicroscopy experiments. Possibly this mechanism contributes to the formation of hyalinosis lesions which in other models also have been shown to develop at the sites of podocyte detachment [40].

In conclusion, this study demonstrates that direct damage to podocytes without ultrastructural changes of the basement membrane, the endothelial and the mesangial cells leads to *in vivo* single nephron albuminuria. As podocytes seem to be crucial in regulating the permeability to albumin of the GCW, further studies of the dynamic interaction between podocytes and the basement membrane will be of great clinical importance to elucidate the precise mechanisms of this process.

Acknowledgments

Dr. Wim Laurens is a Research Assistant from the Belgian National Fund for Scientific Research. Daniela Cavallotti provided excellent technical assistance for the electron microscopy study. Lydie Vanrykel prepared the immunogold electron microscopy sections. Procedures involving animals and their care are conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec.12,1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985.) Reproduction of color figures was made possible by a grant from Zeneca Pharmaceuticals, Belgium.

Reprint requests to Wim Laurens, M.D., Department of Nephrology, Catholic University of Leuven, University Hospital Gasthuisberg, Herestraat, 49, B-3000 Leuven, Belgium.

References

1. KANWAR YS, ROSENZWEIG LJ: Altered glomerular permeability as a result of focal detachment of the visceral epithelium. *Kidney Int* 21:565–574, 1982
2. DANIELS BS, DEEN WM, MAYER G, MEYER T, HOSTETTER TH: Glomerular permeability barrier in the rat: Functional assessment by in vitro methods. *J Clin Invest* 92:929–936, 1993
3. BROYER M, MEYRIER A, NIAUDET P, HABIB R: Minimal change and focal segmental glomerular sclerosis, in *Oxford Textbook of Nephrology*, edited by CAMERON S, DAVIDSON AM, GRÜNFELD JP, KERR D, RITZ E, Oxford, Oxford University Press, 1992, pp 298–339
4. LAURENS WEAA, VANRENTERGHEM YFC, STEELS PSJ, VAN DAMME BJC: A new single nephron model of focal and segmental glomerulosclerosis in the Munich-Wistar rat. *Kidney Int* 45:143–149, 1994
5. REMUZZI A, PUNTORIERI S, MAZZOLENI A, REMUZZI G: Sex related differences in glomerular ultrafiltration and proteinuria in Munich-Wistar rats. *Kidney Int* 34:481–486, 1988
6. REMUZZI A, PUNTORIERI S, ALFANO M, MACCONI D, ABBATE M, BERTANI T, REMUZZI G: Pathophysiologic implications of proteinuria in a rat model of progressive glomerular injury. *Lab Invest* 67:572–579, 1992
7. REMUZZI A, PUNTORIERI S, BRUGNETTI B, BERTANI T, REMUZZI G: Renoprotective effect of low iron diet and its consequence on glomerular hemodynamics. *Kidney Int* 39:647–652, 1991
8. READ SM, NORTHCOTE DH: Minimization of variations in the response to different proteins of the Coomassie blue G dye-binding assay for protein. *Anal Biochem* 116:53–64, 1981
9. LAEMNLI UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970
10. MARANTO AR: Neuronal mapping: A photooxidation reaction makes Lucifer Yellow useful for electron microscopy. *Science* 217:953–955, 1982
11. SANDELL JH, MASLAND RH: Photoconversion of some fluorescent markers to a diaminobenzidine product. *J Histochem Cytochem* 36: 555–559, 1988
12. SCHMUED LC, SNAVELY LF: Photoconversion and electron microscopy localization of the fluorescent axon tracer Fluoro-Ruby. *J Histochem Cytochem* 41:777–782, 1993
13. DE VOS R, DE WOLF-PEETERS C, VAN DEN OORD JG, DESMET VJ: A recommended procedure for ultrastructural immnohistochemistry on small human tissue samples. *J Histochem Cytochem* 33:959–964, 1985
14. TIMMS BG: Post-embedding immunogold labeling for electron microscopy using LR white resin. *Am J Anat* 175:267–275, 1986
15. FERNANDO NS, BOYCE NW, HOLDWORTH SR, NEALE TJ: Localization of antiglomerular basement membrane binding polyclonal antibodies in rat lung and kidney using post-embedding immunogold electron microscopy. *Br J Exp Pathol* 70:557–565, 1989
16. SCALA C, CENACCHI G, FERRARI C, PASQUINELLI G, PREDA P, MANARA GC: A new acrylic resin formulation: A useful tool for histological ultrastructural and immunocytochemical investigations. *J Histochem Cytochem* 40:1799–1804, 1992
17. DRUMOND MC, DEEN WM: Structural determinants of glomerular capillary hydraulic permeability. (abstract) *J Am Soc Nephrol* 3:560, 1992
18. GHITESCU L, DESJARDINS M, BENDAYAN M: Immunocytochemical study of glomerular permeability to anionic, neutral and cationic albumins. *Kidney Int* 42:25–32, 1992
19. FUJIGAKI Y, NAGASE M, KOBAYASHI S, HIDAKA S, SHIMOMURA M, HISHIDA A: Intra-GBM site of the functional filtration barrier for endogenous proteins in rats. *Kidney Int* 43:567–574, 1993
20. BRENDEL K, MEEZAN E, NAGLE RB: The acellular perfused kidney: A model for basement membrane permeability, in *Proceedings of the First International Symposium on the Biology and Chemistry of Basement Membranes*, edited by KEFALIDES NA, New York, Academic Press, 1978, pp 177–193
21. WHITESIDE C, PRUTIS K, CAMERON R, THOMPSON J: Glomerular epithelial detachment, not reduced charged density, correlates with proteinuria in adriamycin and puromycin nephrosis. *Lab Invest* 61: 650–660, 1989
22. SAMATA K, KIMURA T, SATOH S, WATANABE H: Chemical removal of the endothelium by saponin in the isolated dog femoral artery. *Eur J Pharmacol* 128:85–91, 1986
23. WIEST E, TRACH V, DÄMMGEN J: Removal of endothelial function in coronary resistance vessels by saponin. *Basic Res Cardiol* 84:469–478, 1989
24. LEGAN E, SISSON JA: Method to denude rat aortic endothelium with saponin for phosphoinositide analysis in vascular smooth muscle. *J Pharmacol Meth* 23:31–39, 1990
25. MEEZAN E, HJELLE JT, BRENDEL K: A simple, versatile, non-disruptive method for the isolation of morphologically and chemically pure basement membranes from several tissues. *Life Sci* 17:1721–1732, 1976
26. HOUSER MT, SCHEINMAN JI, BASGEN J, STEFFES MW, MICHAEL AF: Preservation of mesangium and immunohistochemically defined antigens in glomerular basement membrane isolated by detergent extraction. *J Clin Invest* 69:1169–1175, 1982
27. SALANT DJ, MADAIO MP, ADLER S, STILMANT MM, COUSER WG: Altered glomerular permeability induced by F(ab')2 and Fab' antibodies to rat renal tubular epithelial antigen. *Kidney Int* 21:36–43, 1981
28. NATORI Y, HAYAKAWA I, SHIBATA S: Passive Heyman nephritis with acute and severe proteinuria induced by heterologous antibody against renal tubular brush border glycoprotein gp108. *Lab Invest* 55:63–70, 1986
29. ORIKASA M, MATSUI K, OITE T, SHIMUZU F: Massive proteinuria induced in rats by a single intravenous injection of a monoclonal antibody. *J Immunol* 141:807–814, 1988
30. MENDRICK DL, RENNKE HG: I. Induction of proteinuria in the rat by a monoclonal antibody against SGP-115/107. *Kidney Int* 33:818–830, 1988
31. O'MEARA YM, NATORI Y, MINTO AWM, GOLDSTEIN DJ, MANNING EC, SALANT DJ: Nephrotoxic antiserum identifies a β1-integrin on rat glomerular epithelial cells. *Am J Physiol* 262:F1083–F1091, 1992
32. ASSMANN KJM, VAN SON JPHF, DIJKMAN HBPM, KOENE RAP: A nephritogenic rat monoclonal antibody to mouse aminopeptidase A. Induction of massive albuminuria after a single intravenous injection. *J Exp Med* 175:623–635, 1992

33. ADLER S, CHEN X: Anti-Fx1A antibody recognizes a β1-integrin on glomerular epithelial cells and inhibits adhesion and growth. *Am J Physiol* 262:F770–F776, 1992
34. DRENCKHAHN D, FRANKE RP: Ultrastructural organisation of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat, and man. *Lab Invest* 59:673–682, 1988
35. SHAKE JG, BRANDT RC, DANIELS BS: Angiotensin II induces actin polymerisation within the glomerular filtration barrier: Possible role in the local regulation of ultrafiltration. (abstract) *J Am Soc Nephrol* 3:568, 1992
36. SHARMA R, LOVELL HB, WIEGMANN TB, SAVIN VJ: Vasoactive substances induce cytoskeletal changes in cultured rat glomerular epithelial cells. *J Am Soc Nephrol* 3:1131–1138, 1992
37. REMUZZI A, PUNTORIERI S, BATTAGLIA C, BERTANI T, REMUZZI G: Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. *J Clin Invest* 85:541–549, 1990
38. LAFAYETTE RA, MAYER G, PARK SK, MEYER TW: Angiotensin II blockade limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 85:541–549, 1992
39. KRIZ W, HACKENTHAL E, NOBILING R, SAKAI T, ELGER M: A role for podocytes to counteract capillary wall distension. *Kidney Int* 45:369–376, 1994
40. FRIES JWU, SANDSTROM DJ, MEYER TW, RENNKE HG: Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest* 60:205–218, 1989