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Structural continuity of filtration slit (slit diaphragm) to plasma membrane of podocyte

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Structural continuity of filtration slit (slit diaphragm) to plasma membrane of podocyte. Murine monoclonal antibody 5-1-6 was reported to bind to the slit membrane and closely related structures in rat renal glomeruli; it induced heavy, reversible proteinuria and appeared to redistribute onto the plasma membrane of epithelial cells after binding at the original target sites. This phenomenon of antigenic movement has not been analyzed in detail to date. In addition to normal kidneys we also studied localization of the antigen recognized by monoclonal antibody 5-1-6 in protamine sulfate-perfused rat kidneys, in which slit diaphragms are known to be functionally modified. Isolated glomeruli as well as ultrathin kidney cryosections were labeled by the immunogold technique to clarify the relation between this antigen and the slit diaphragm. Sequential localization of injected monoclonal antibody was visualized using a post-embedding immunogold method in rats 2 hours to 12 days after injection of antibody. Ultrastructural immunogold labeling demonstrated that under normal conditions antigenic molecules were expressed mainly in the area beneath the slit diaphragms. Occasionally labeling was found at the base of the foot process, facing the glomerular basement membrane. After protamine sulfate treatment antigenic sites were dislocated due to the lifting and disruption of slit diaphragms, indicating that this antigen is associated with slit diaphragms. Injected antibody was localized at the filtration slits at 2 hours, and by 12 hours it had moved onto the apical plasma cell membrane of foot process. In addition, from 3 days onwards patch or cap-like formation on the plasma cell membrane of podocytes was seen. Possible shedding of antibody from podocyte cell surface membrane was occasionally encountered, but internalization of antibody was a minor event. Elution experiments in isolated glomeruli at day 3 indicated that antigen and antibody were both localized on the podocyte cell surface membrane, suggesting redistribution of immune complexes. In conclusion, filtration slits (slit diaphragms) and the apical membrane of foot process of podocytes demonstrate structural continuity, as revealed by the movement of the antigen recognized by monoclonal antibody 5-1-6 as antigen-antibody complexes.

The podocyte is the most highly differentiated cell in the renal glomerulus. The narrow spaces between the foot processes of podocytes have the form of long, meandering channels, the so-called filtration slits. This filtration slit is bridged by a diaphragm, referred to as the slit diaphragm [1]. Under pathological conditions, retraction and flattening of foot processes of podocytes occur, associated with dislocation of the slit diaphragm [2–4]. These structures are believed to regulate hydraulic conductivity and water flow [5–7], although the exact function of the slit diaphragm and its relationship to the podocyte cell surface membrane are not well-understood.

Some years ago, Orikasa et al [8] described a monoclonal antibody (MoAb) 5-1-6, which appears to recognize a 51 kDa protein of rat slit diaphragms, and which induces heavy, reversible proteinuria after a single intravenous injection. However, it is not clear whether this antigen is also expressed on the surface membrane of foot process [8]. In initial studies [8, 9] using the immunoperoxidase technique, intravenously injected antibody was detected at first on the foot process surface membrane, mainly on slit diaphragms, later on restricted areas of the epithelial cell surface and in multivesicular bodies of epithelial cells, suggesting redistribution of the antibody onto the epithelial cell surface. This phenomenon, presumably reflecting antigenic movement, has not been analyzed in detail. Kinetic studies of immune complex formation may provide a clue to its functional relevance for the integrity of slit diaphragms and foot processes and for regulation of glomerular permeability.

We undertook the present study, using immunogold labels, to clarify the exact localization of this antigen. In addition, we studied the fate of antigen-antibody complexes formed after intravenous injection of MoAb 5-1-6. We demonstrated that this antigen is closely associated with the slit diaphragm and that the intravenously injected antibody moved from the filtration slit onto the apical plasma cell membrane of podocytes, probably as antigen-antibody complexes. This strongly suggests that the filtration slits (slit diaphragms) are in structural continuity with the apical plasma membrane of the foot process.

Methods

Animals

Male wistar rats (120 to 150 g body weight, Harlan Winkelmann, Borchen, Germany and Japan SLC Co., Shizuoka, Japan) were used throughout this study.

Localization of antigen recognized by MoAb 5-1-6 in normal and protamine sulfate-treated rat glomeruli

In preliminary studies localization of the antigen recognized by MoAb 5-1-6 could not be satisfactorily visualized using a post-embedding immunogold technique with Lowicryl K4M (Polysciences, Ltd., Eppelbeim, Germany) or Unicryl resins (British

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BioCell, UK). This antigen is believed to be a glomerular epithelial cell surface membrane associated protein [8]; therefore, the following procedure using isolated glomeruli [10] was adopted. In order to exclude the possibility of redistribution of the antigen during isolation of glomeruli, ultrathin frozen sections of whole renal cortex were also examined.

Immunogold labeling of isolated glomeruli. Rats were anesthetized with ether and the abdomen was opened through a midline incision. A 27 gauge needle was inserted into the aorta below the junction of the left renal artery. Shortly after perfusion was started, the aorta was clamped above the renal vessels and the inferior vena cava was cut. In 3 rats phosphate buffered saline (PBS) containing protease inhibitors (5 mM sodium tetrathionate, 5 mM benzamidine, 5 mM EDTA and 5 M leupeptin) was perfused for 3 minutes at 120 mm Hg. In order to induce dislocation of slit diaphragms [11, 12], in another 3 rats Hanks' balanced salt solution (HBSS) at 37°C was first perfused for 3 minutes, followed by HBSS containing 500 μg/ml of protamine sulfate (type V, Sigma Chemical Co. St. Louis, MO, USA) at 37°C for 10 minutes, then PBS containing protease inhibitors for 3 minutes at 120 mm Hg. A portion of renal cortex from each rat was processed for immunofluorescent study. Renal glomeruli were isolated from the remainder of the kidney by sequential sieving. The isolated glomeruli were decapsulated by means of several passages through a 26 gauge needle. Glomeruli were fixed with 2% periodate-lysine-paraformaldehyde (PLP) [13] for 2 hours at room temperature, incubated with MoAb 5-1-6 (spent culture supernatant, diluted 1:50 in 10% normal goat serum), or MoAb RVG1 as an IgG subclass-matched control overnight at 4°C, then incubated with rabbit anti-mouse IgG (preabsorbed with rat serum, diluted 1:500 in 10% normal goat serum, Dianova Immunotech GmbH, Hamburg, Germany) for 5 hours at 4°C, followed by incubation with goat anti-rabbit IgG tagged with 10 nm or 5 nm colloidal gold (diluted 1:10 in 10% normal goat serum, Amersham, Frankfurt, Germany) for 5 hours at 4°C. PBS was used for washing glomeruli between each step. After completion of the incubation procedure glomeruli were fixed with 1% glutaraldehyde overnight, then post-fixed with 1% osmium tetroxide for 2 hours. Embedding was done in Epon by routine methods, and ultrathin sections were stained with uranyl acetate and examined in a Zeiss EM10A electron microscope.

Immunogold labeling of ultrathin cryosections. Two further groups of rats were treated with (N = 2) or without (N = 2) protamine sulfate as described above. The kidneys were removed and fixed with 2% PLP solution for 2 hours at room temperature, after which they were immersed in 1.8 M sucrose in phosphate buffer containing 20% polyvinylpyrrolidone, and frozen in liquid nitrogen. Ultrathin cryosections were cut at −120°C on a Reichert Ultracut equipped with an FC-4E cryoattachment, following the technique of Tokuyasu [14]. Sections were transferred to 200-mesh nickel grids, which had been coated with formvar and carbon. After quenching with 10% fetal calf serum containing 0.02 M glycine, the sections were incubated for 2 hours with MoAb 5-1-6 (spent culture supernatant) or MoAb RVG1 as a control and with rabbit anti-mouse IgG (diluted 1:500) for 30 minutes, followed by goat anti-rabbit IgG tagged with 10 nm gold (diluted 1:10) for 30 minutes. Sections were then post-fixed with 2% glutaraldehyde for 10 minutes, stained with 2% uranyl acetate for 10 minutes, then adsorption-stained with 2% polyvinyl alchol containing 0.2% uranyl acetate for 5 minutes, and examined in a JEM-200CX electron microscope (JEOL, Tokyo, Japan).

Sequential localization of injected MoAb 5-1-6 in glomeruli

Rats were injected with 4 mg of MoAb 5-1-6 (prepared from ascites of *Balb/c* mice by 50% ammonium sulfate precipitaion) in 1 ml PBS intravenously under ether narcosis. Sixteen-hour urine was collected at days 1, 3, 8 and 12 and protein content was measured by the biuret method [15]. Two (N = 3), 12 (N = 2), 24 hours (N = 2), and 3 (N = 3), 8 (N = 3) and 12 days (N = 2) after antibody injection kidneys were flushed with PBS. A portion of the kidney was processed for immunofluorescent study and the remainder of the renal cortex was fixed with 2% PLP solution for 2 hours at room temperature. PLP fixed cortical tissues were dehydrated in a graded ethanol series and embedded in Unicryl (British BioCell, UK) at −30°C according to the manufacturer's protocol. Ultrathin sections were incubated with either donkey anti-mouse IgG tagged with 12 nm colloidal gold (diluted 1:40 in Tris-HCl buffer, preabsorbed with rat serum, Dianova) or rabbit anti-mouse IgG (diluted 1:500 in 0.02 M Tris-HCl buffer, pH 7.0 containing 1% bovine serum albumin, Dianova) and subsequently with donkey anti-rabbit IgG tagged with 12 nm colloidal gold (diluted 1:40 in Tris-HCl buffer, Dianova). As controls, rats were injected with 4 mg of MoAb RVG1 in 1 ml PBS, and sacrificed at 2 hours (N = 2), 3 (N = 2) and 8 days (N = 2) and kidneys were processed as above. As histological controls, the first antibody was omitted or normal rabbit IgG was used in its place. Sections were stained with uranyl acetate, and examined in the electron microscope.

Co-localization of Ag-Ab complexes on the outer plasma membrane of the glomerular epithelial cell

Rats studied 3 days (N = 4) after MoAb 5-1-6 injection were selected for this purpose, because the existence of enough antigenic molecules to be visualized on the epithelial cell surface was expected according to the labeling density of bound MoAb 5-1-6 on the epithelial cell surface. Isolated glomeruli were prepared from rats as described above, using borate buffer (pH 8.0) instead of PBS. Glomerular bound MoAb 5-1-6 was eluted from isolated glomeruli by using ImmunoPureR Gentle Ag/Ab Elution Buffer, pH 6.5 (Pierce, The Netherlands) at room temperature for 30 minutes with frequent vortexing. After washing glomeruli three times (5 minutes, 1000 rpm) with borate buffer, then three times (5 minutes, 1000 rpm) with PBS, a part of the glomeruli were embedded in OCT compound (Miles Inc. IN, USA) and processed for immunofluorescent study. The rest of the glomeruli were fixed with 2% PLP for 2 hours, incubated with biotinylated MoAb 5-1-6 or biotinilated MoAb RVG1 as a control (labeled by means of a biotinylation kit, Amersham) overnight, then incubated with streptavidin-10 nm colloidal gold complexes (Diluted 1:10 in PBS, Amersham) for 5 hours. After completion of these steps, post-fixation, embedding and observation were performed, following the same protocol as that described above for immunogold labeling of isolated glomeruli.

Immunofluorescence

Kidney blocks or isolated glomeruli embedded in OCT compound were quick-frozen in n-hexane cooled to −70°C and cryostat sections (4 μm) were cut. For normal and protamine

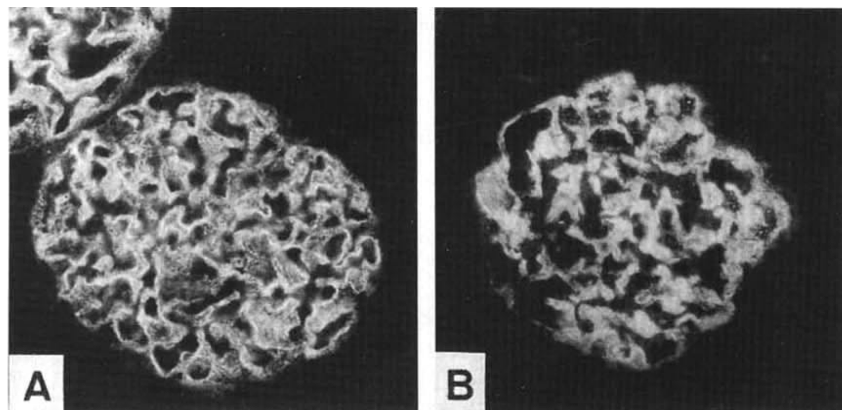

Fig. 1. *Indirect immunofluorescent findings related to the antigen recognized by MoAb 5-1-6 in normal* (**A**) *and protamine sulfate-perfused rat kidneys* (**B**). Linear to fine granular staining along the capillary wall is observed in normal kidneys (A, B), and protamine sulfate-treated kidneys (B) reveal diminished linear staining and a more prominent fine granular pattern. Magnification × 400.

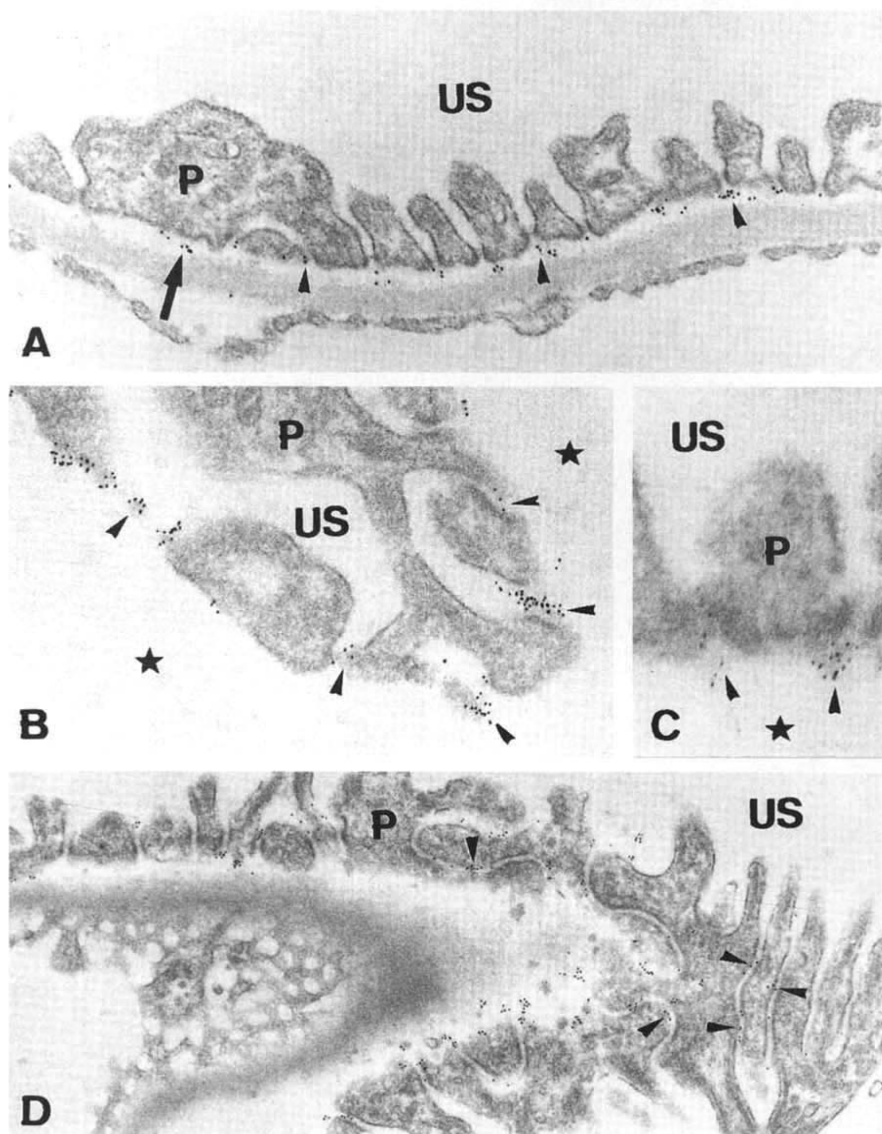

Fig. 2. *Localization of antigen recognized by MoAb 5-1-6 by an indirect immunogold electron microscopy on isolated glomeruli in normal rat.* **A.** The gold particles (10 nm), which indicate the binding sites of MoAb 5-1-6, are localized mainly in the filtration slits, beneath the slit diaphragms in cross-section of the glomerular capillary wall. Gold particles are occasionally found at the base of foot process facing the GBM (arrow). The cell surfaces of podocytes within the urinary space are devoid of labeling. **B.** In the epithelial foot processes detached during processing, gold particles (10 nm) are much more concentrated beneath the slit diaphragms than in intact glomerular capillary wall (A). **C.** Five nm gold particles also show the same distribution of antigenic sites. **D.** In tangential section gold particles (10 nm) are found at interdigitating areas of foot processes (arrow heads). Abbreviations are: P, podocyte; US, urinary space. Asterisks indicate areas of detachment of the epithelial foot processes from the GBM during tissue processing. Magnifications are: A, × 35,000; B, × 38,000; C, × 92,000; D, × 24,000.

sulfate-treated rats, sections were stained indirectly with MoAb 5-1-6 (spent culture medium) subsequently using fluorescein isothiocyanate (FITC) labeled anti-mouse IgG (preabsorbed with rat serum, Dianova). For rats injected with MoAb 5-1-6 sections were stained directly with FITC labeled anti-mouse IgG, FITC labeled anti-rat IgG (Nordic Immunological Laboratories,

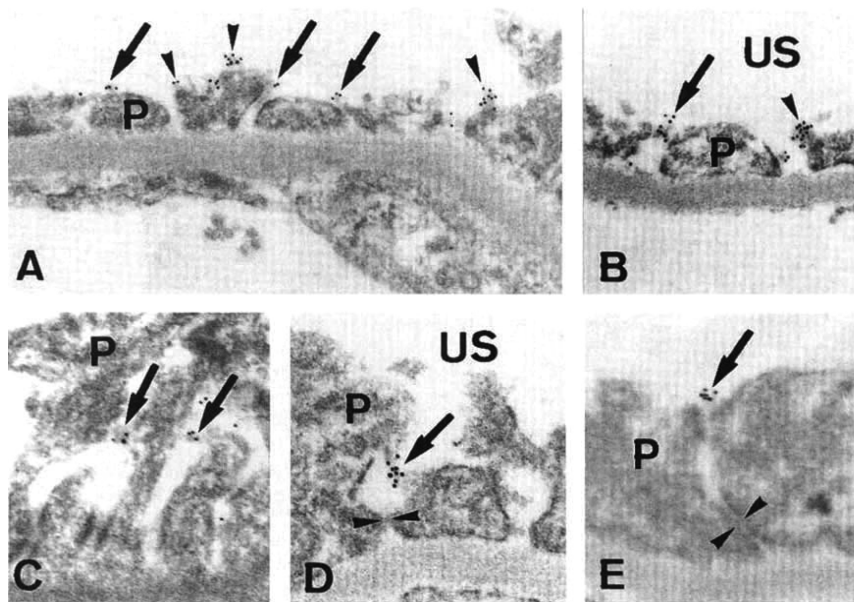

Fig. 3. *Localization of antigen recognized by MoAb 5-1-6 by an indirect immunogold electron microscopy on isolated glomeruli in rats, perfused with protamine sulfate (500 μg per ml) for 10 minutes.* Gold particles (10 nm) can be seen associated with the dislocated slit diaphragms (**A, B, C, D, E**) (arrows), and around amorphous material at apical parts of foot processes (A, B) (arrow heads). The occluding-type junctions are not labeled (D, E) (arrow heads). Abbreviations are: P, podocyte; US, urinary space. Magnifications are: A, × 34,000; B, × 31,000; C, × 38,000; D, × 50,000; E, × 44,000.

Kempten, Germany), and FITC labeled anti-rat C3 (Cappel Laboratories, Cochraneville, PA, USA), respectively. Sections from isolated glomeruli at day 3 after elution of bound MoAb 5-1-6 were stained directly with FITC labeled anti-mouse IgG, or stained indirectly with biotinylated MoAb 5–1-6 and FITC labeled streptavidin (Sigma) subsequently. As histological controls, biotinylated MoAb 5-1-6 was omitted or biotinylated MoAb RVG1 was used instead of biotinylated MoAb 5-1-6.

Results

Antigenic molecule recognized by MoAb 5-1-6 is closely associated with the slit diaphragm

The *in vitro* staining pattern of MoAb 5-1-6 in normal rats, by indirect immunofluorescence, was of linear staining to fine granular pattern along the glomerular capillary wall (Fig. 1A). Protamine sulfate-treated rat kidneys revealed diminished linear staining and a more prominent fine granular pattern (Fig. 1B). Staining intensity by immunofluorescence was almost the same in rats with or without protamine sulfate-treatment.

By immunoelectron microscopy on sections of glomeruli isolated from normal rats, gold particles, representing the antigen, were localized mainly in the filtration slit, beneath the slit diaphragm (Fig. 2A). Occasionally gold particles were found at the base of the foot processes, facing the glomerular basement membrane (Fig. 2A). Exposed surfaces of glomerular epithelial cells were devoid of labeling (Fig. 2A). In the cases where the epithelial cells had been detached from the glomerular basement membrane during tissue processing, most antigen was left at the site of the foot processes (Fig. 2B, C), indicating that this antigen is a constituent of the slit diaphragm or the epithelial cell surface membrane. In detached epithelial foot processes gold particles were much more concentrated beneath the slit diaphragms than in intact glomerular capillary walls (Fig. 2B). In tangential sections gold particles were found at interdigitating areas of foot processes (Fig. 2D), indicating that this antigen is a component of the filtration slit (slit diaphragm).

After treatment with protamine sulfate, many slit diaphragms were lifted [12] and gold particles were found associated with the dislocated slit diaphragms (Fig. 3). Gold labeling was also found around amorphous material seen at apical parts of foot processes (Fig. 3A, B), which lacked slit diaphragms, suggesting that slit diaphragms had been disrupted or washed away. At areas of occluding-type junctions between foot processes, almost no labeling was found (Fig. 3D, E). Labeling density of gold particles was in general more sparse than that in normal rats, probably due to the limitated accessibility of immunomarkers.

Findings in the glomeruli by immunogold labeling of ultrathin cryosections kidneys with (Fig. 4C, D, E) or without (Fig. 4A, B) protamine sulfate-treatment were almost the same as that of immunogold labeling of isolated glomeruli from similar experiments (Fig. 2, 3).

Movement of in vivo *administered MoAb 5-1-6*

Proteinuria started as early as day 1 (48.9 ± 13.8 mg/day, means ± SD) after MoAb 5-1-6 injection, increased at day 3 (103.6 ± 10.7 mg/day), and peaked at day 8 (129.3 ± 13.1 mg/day), then decreased at day 12 (39.4 mg/day). No abnormal proteinuria was observed in control rats or rats prior to MoAb injection. By immunofluorescent study, linear binding of mouse IgG along the glomerular capillary wall was found from 2 to 24 hours after MoAb 5-1-6 injection (Fig. 5A); at day 3 it changed to a fine granular pattern (Fig. 4B) and afterwards became more scattered (Fig. 5C). In control rats staining for mouse IgG was negative and in all control and experimental animals rat IgG and rat C3 were negative. These urinary and immunofluorescent findings are comparable to those already reported [8]. By immunoelectron microscopy, the binding pattern of *in vivo* administered MoAb 5-1-6, shown by gold particles, at 2 hours was essentially similar to that seen *in vitro* (Fig. 6A). Most gold particles were accumulated at the filtration slits (Fig. 6A) and they were only occasionally found within the foot processes. After 12 hours, solitary or a low number of grouped gold particles were encountered on the apical

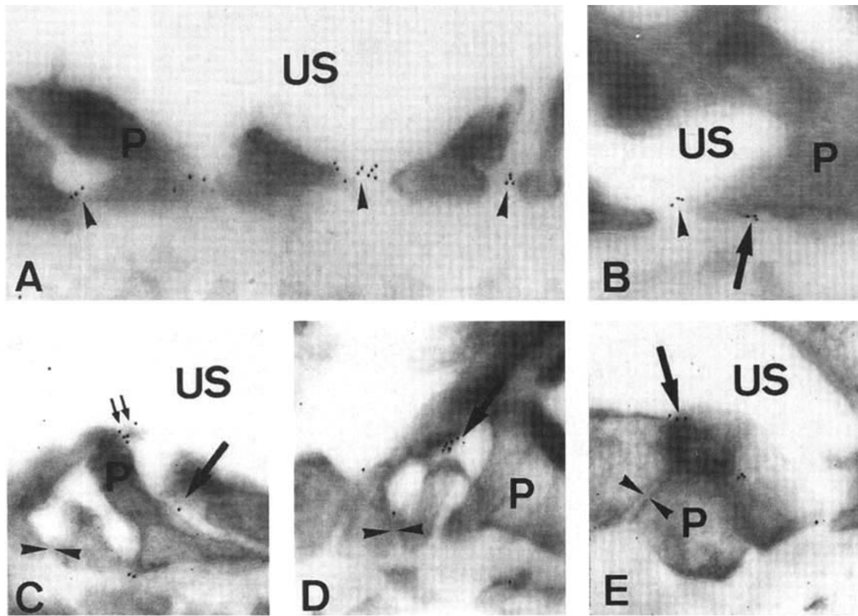

Fig. 4. *Glomerular localization of antigen recognized by MoAb 5-1-6 on ultrathin cryosections in normal (**A, B**) and protamine sulfate-perfused rats (**C, D, E**) by indirect immunogold electron microscopy.* In normal glomeruli (A, B) the gold particles (10 nm) (arrow heads) are localized in the filtration slits, and labeling is seen occasionally at the base of foot processes facing the GBM (arrow). In protamine sulfate-treated glomeruli (C, D, E) gold particles can be seen associated with the dislocated slit diaphragms (C, D, E) (arrows) and around amorphous material at apical parts of foot process (C) (double arrow). The occluding-type junctions are not labeled (C, D, E) (arrow heads). Abbreviations are P, podocyte; US, urinary space. Magnification for A, B, C, and D is: × 32,000; Magnification for E is: × 36,000.

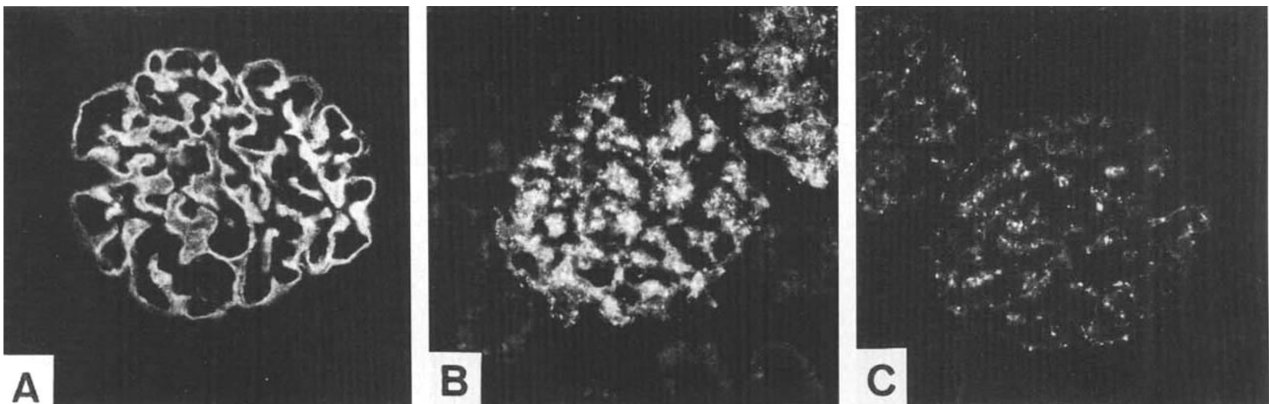

Fig. 5. *Direct immunofluorescent findings related to injected MoAb 5-1-6 at 2 hours (**A**), 3 (**B**) and 8 (**C**) days after administration of MoAb 5-1-6.* **A.** Linear binding of mouse IgG along the glomerular capillary wall is observed. **B.** A fine granular staining pattern can be seen along the glomerular capillary wall. **C.** The staining pattern becomes more sparser. Magnification × 400.

plasma membrane of the foot processes (Fig. 6B, C). The frequency of gold particles within the podocytes was slightly increased (Fig. 6C). From days 3 to 12, gold particles were lined up on the outer plasma cell membrane of podocytes, patch or cap-like formation could be frequently seen (Fig. 6D, E, F, I), and gold particles in filtration slits decreased with time. Although pictures which speak for shedding of antibody from podocyte cell surface membrane were occasionally seen in these periods, we could not completely exclude that these areas might be due to tangential sections of the plasma membrane (Fig. 6G, H, I). Patterns which suggested that antibody is being taken up into podocytes from the cell surface were not found, and gold particles within podocytes did not increase after 3 days; therefore, internalization seemed to be minor. Only a very limited partial retraction of the foot process was observed during the experimental period.

Antigenic molecule recognized by MoAb 5-1-6 is redistributed as antigen-antibody complexes

By immunofluorescent study, neutral elution buffer treated isolated glomeruli 3 days after MoAb 5-1-6 injection showed almost negative staining for mouse IgG (Fig. 7A); however, they showed positive granular staining pattern for MoAb 5–1-6 with the biotin-streptavidin system (Fig. 7B). Glomerular staining was negative with histological controls under all conditions tested. By immunoelectron microscopy, gold particles, indicating antigenic molecules, were found on the apical plasma cell membrane of podocytes as well as in filtration slits (Fig. 7C, D, E). This is comparable to the labeling pattern of injected MoAb 5–1-6 at day 3, strongly suggesting that antigenic molecules were redistributed from the filtration slit (slit diaphragm) to the apical plasma cell membrane of podocytes as antigen-antibody complexes *in vivo.*

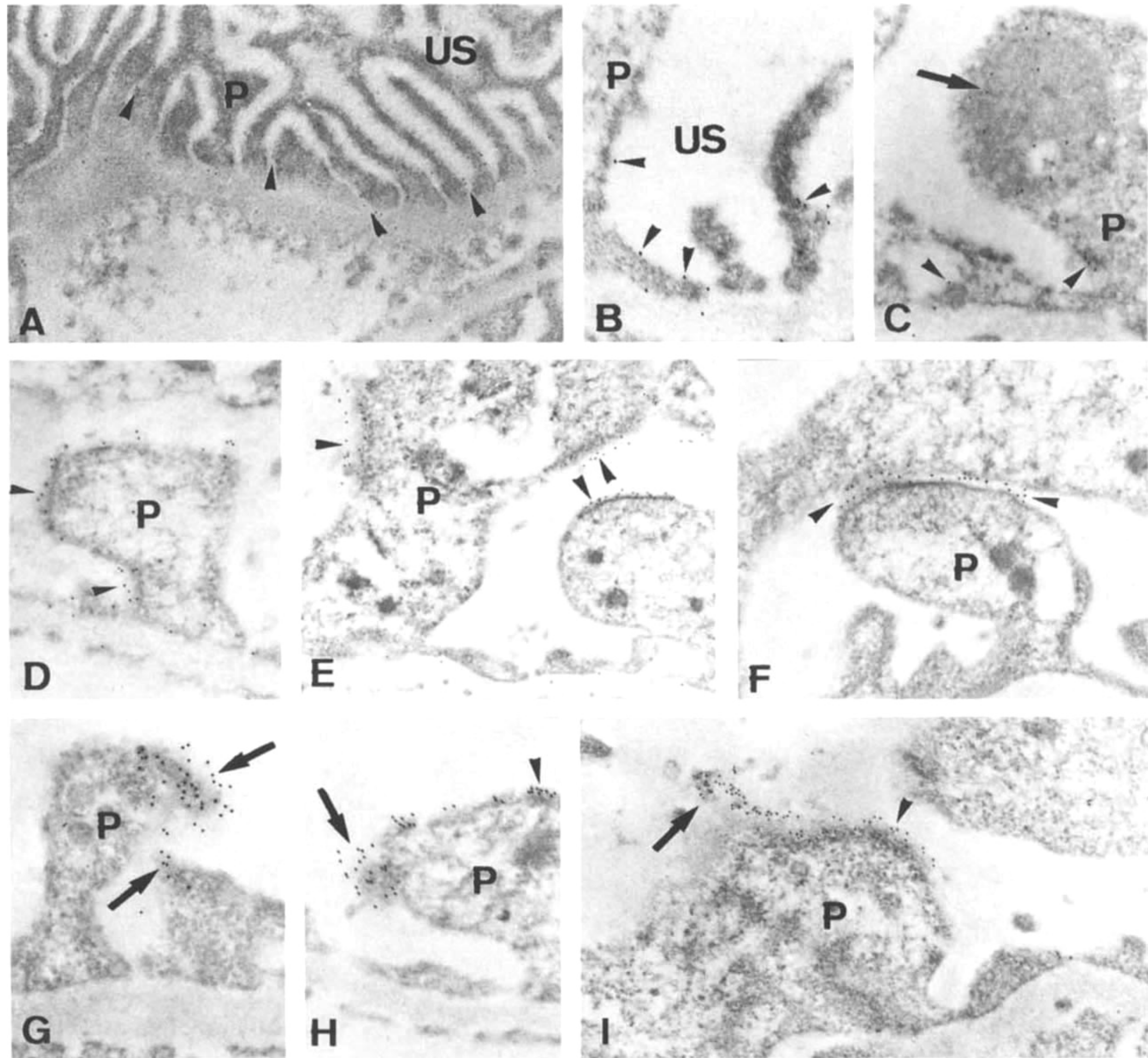

Fig. 6. *Localization of injected MoAb 5-1-6 by postembedding immunogold (12 nm) electron microscopy at 2 (**A**), 12 (**B**), 24 hours (**C**), and 3 (**D, E, F, G, H**) and 8 days (**I**) after injection of MoAb 5-1-6.* A. In tangential section. Gold particles, indicating mouse IgG, are located mainly in filtration slits (arrows). B and C. Solitary or a low number of gold particle are seen on the apical plasma membrane of the foot processes (arrow heads). Gold particles are seen within the podocyte (C) (arrow). D. Gold particles can be seen continuously from filtration slit to the apical plasma membrane of foot process as cluster (patch-like) formation (arrow heads). E and F. Gold particles lined up on the outer plasma cell membrane of podocytes as patch or cap-like formation can be seen (arrow heads). G, H and I. Gold particles are probably shed from podocyte cell surface membrane (arrows). Abbreviations are: P, podocyte; US, urinary space. Magnifications are: A, × 38,000; B, × 35,000; C, × 28,000; D, × 30,000; E, × 19,000; F, × 26,000; G, × 37,000; H, × 33,000; I, × 24,000.

Experiments performed to assess the specificity of the labeling by using biotinilated MoAb RVG1 showed little labeling over glomeruli.

Discussion

In the present study, we analyzed in detail the location of antigen recognized by MoAb 5-1-6 and the fate of antigen-antibody complexes formed after intravenous injection of MoAb 5-1-6. The conflicting findings between the current study, using immunogold labels and a previous study, using an immunoperoxidase label [8], may result from the different techniques used. Presumably, a diffusion artifact of the reaction product influenced the results of the immunoperoxidase study.

The present ultrastructural study employed immunogold labeling on isolated glomeruli as well as ultrathin cryosectioning and demonstrated that antigenic molecules recognized by MoAb 5-1-6 were mainly localized in the filtration slit, beneath the slit diaphragm, but not on the surface of podocytes within the urinary space. These findings confirm previous observations obtained by using immunogold labeling on ultrathin cryosections [8], but contradict data obtained using the immunoperoxidase technique, the latter suggesting the existence of this particular antigen on the

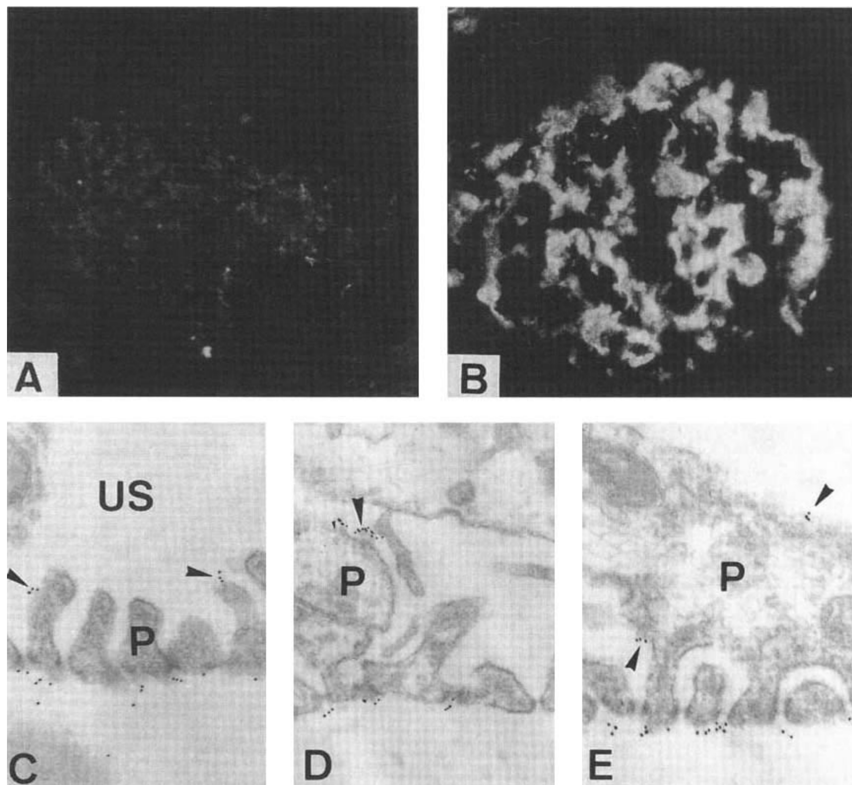

Fig. 7. *Findings in isolated glomeruli from rats 3 days after administration of MoAb 5-1-6, in which bound MoAb 5-1-6 is eluted.* **A.** Direct immunofluorescent staining for mouse IgG is almost negative in isolated glomerulus, showing efficiency of elution. **B.** Indirect immunofluorescent staining for antigenic molecules recognized by MoAb 5-1-6 shows positive granular pattern with biotin-streptavidin system. **C, D** and **E.** Immunoelectron micrograph. Gold particles (10 nm), indicating the localization of antigen recognized by MoAb 5-1-6, can be seen on the apical plasma membrane of podocytes (arrow heads) as well as in filtration slits. Abbreviations are: P, podocyte; US, urinary space. Magnifications are: A, B, × 400; C, × 33,000; D, × 27,000; E, × 37,000.

foot process cell surface as well [8]. The significance of occasional labeling at the base of the foot processes is not clear. This may be the original site of synthesis or a region where the molecules are passively taken up. Data supporting the first possibility were recently reported by Kawachi et al in neonatal rat kidneys [16]; they found antigen along the basal and lateral surface below occluding junctions at the early capillary loop stage.

In protamine sulfate-treated rats, gold particles, demonstrating the reaction site of MoAb 5-1-6, were found to be associated with the dislocated slit diaphragms, indicating that this molecule is actually related to the slit diaphragm. In contrast to normal glomerular structures, after destruction of the slit diaphragms by protamine sulphate-perfusion, gold particles were found at both sites of the slit diaphragms, indicating that the antigen on the urinary site was accessible for the antibody.

The molecular character of the slit diaphragm bridging the filtration slit and connecting the foot processes is still unknown. Recently, Schnabel, Anderson and Farquhar [17] demonstrated that ZO-1 (tight junction protein, 225 kDa) was concentrated along the cytoplasmic surface of the slit diaphragms of normal rats, suggesting that the slit diaphragm is a variant of the tight junction. Subsequent studies by this group [18] showed that in protamine sulfate-treated rats, ZO-1 was concentrated along both the newly found occluding-type junctions and the dislocated slit diaphragms, supporting the above concept. It seems unlikely that the so-called tight junction possesses the antigen recognized by MoAb 5-1-6 that may only make up the unique structure and function of the slit diaphragm.

In sections from normal, isolated rat glomeruli there is some space between the slit diaphragm and the gold particles. (This distance seems to be too great to represent merely the dimensions of immunomarkers which we used here.) Taking the concept of an association of this antigen with slit diaphragms into consideration, two explanations are possible: (1) the antigen is a substructure of the slit diaphragm (not visible by electron microscopy) which is located below the slit diaphragm; (2) the antigen is a constituent of the slit diaphragm (visible by electron microscopy), but there are some structures beneath the slit diaphragm which hinder the access of immunomarkers and "stretch" them.

The phenomenon of molecular redistribution on plasma membranes by specific ligands is well-known [19–25]. In this study we observed that administered MoAb first bound the antigen localized at the filtration slit, then moved onto the apical plasma membrane of podocytes as an antigen-antibody complex, where antigen is not originally localized. Some antibody or antigen-antibody complexes seemed to be endocytosed by epithelial cells, but antigen-antibody complexes were probably shed from the cell surface directly into the urinary space, rather than being first internalized and then exocytosed. This is consistent with the findings that the amount of antigenic molecules in the proteinuric stage of this model decreases [26]. *In vitro* studies on the redistribution of cell surface antigens have revealed that this process is temperature-dependent, and requires energy, divalent ligands and cytoskeletal activity [19, 20, 23, 24]. In this model, cross-linking of 5–1-6 antigen by divalent MoAb 5-1-6 is necessary to induce proteinuria as well as change the linear pattern to a

granular pattern in immunofluorescence [27]. However, the factors which regulate the kinetics of antigen-antibody complex redistribution on cell membranes in our model are unclear. Certainly chlorpromazine, which is known to inhibit cytoskeletal movement and prevent cap formation of immune complexes [28], did not operate on this phenomenon [9]. The biological significance of this phenomenon is not clear. Our results, that the filtration slit (slit diaphragm) has structural continuity to the apical plasma membrane of podocytes, may be of relevance for this problem.

Recently, we reported using immunogold immunocytochemistry that in *in situ* drip-fixed rat glomeruli the distribution of endogenous albumin and IgG showed a concentration gradient in the glomerular basement membrane, peaking on the inner (endothelial) side [29]. These results suggest that the filtration slit itself does not function as the main filtration barrier for plasma macromolecules under normal conditions. Our preliminary study showed that the concentration gradients of albumin and IgG were less prominent under the proteinuric state in MoAb 5-1-6 administered rats [30]. In this model neither the complement system nor inflammatory cells are involved, and almost no ultrastructural changes were observed [8]. Taking into consideration the role of the filtration slit on regulation of hydraulic conductivity and water flow [5–7], we can hypothesize that in MoAb 5-1-6 administered rats the integrity of the glomerular permselective function in the glomerular basement membrane was probably changed secondarily due to the alteration of hydraulic conductivity. This may have been brought about by disappearance of this antigen from the filtration slit, which was still open, as in the normal state. Analysis of this simple model may provide us with a better understanding of the *in vivo* relationship between hydraulic conductivity and the glomerular permselective function for macromolecules.

The antigen recognized by MoAb 5-1-6 cannot be detected in cultured glomerular epithelial cells, grown from isolated rat glomeruli by immunofluorescent study [31], suggesting that this antigen retains the specific structure and function of glomerular epithelial cells *in vivo.* Moreover, it is of interest that virtually no abnormal structural changes of foot processes were found in rats after intravenous injection of MoAb 5-1-6, as reported here and previously [4], although foot process loss is a characteristic feature during heavy proteinuria. So far it is not clear whether the dislocation of the slit diaphragm is a consequence or a cause of development of the foot process fusion. Characterization of antigenic molecules may help us to further elucidate the structure and function of filtration slit (slit diaphragm).

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