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ORIGINAL PAPER

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Alterations of podocytes in a murine model of crescentic glomerulonephritis

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Abstract Recent observations suggest a central role of podocytes in crescent formation. In experimental glomerulonephritis podocytes disrupt the parietal epithelial layer and attach on its basement membrane, thus forming bridges between the tuft and Bowman's capsule, and they are a major constituent of crescents. In order to explain these findings we hypothesize that inflammation trig- gers motility in podocytes. In the present study we asked whether podocytes display alterations which suggest a migratory behavior in glomerulonephritis. Glomerulonephritis was induced in mice by injection of a rabbit serum against the glomerular basement membrane. The kidneys were perfusion-fixed 6 days later and examined by light and electron microscopy as well as by immunohistochemistry. In glomerulonephritis the apical cytoplasm of podocytes displayed numerous actin-containing microprotrusions. Cortactin, a protein involved in the regulation of actin polymerization, was predominantly expressed in foot processes of podocytes in control mice. It was redistributed to the cell body in glomerulonephritis. In untreated mice β1-integrin was restricted to the foot processes. In glomerulonephritis it was additionally found in the cytoplasm and in the apical cell membrane. Recycling of integrins is a crucial event in initiation of cell migration. ICAM-1 and CD44, the ligation of which induces migratory behaviors, were absent from healthy podocytes but expressed by some podocytes in glomerulonephritis. Thus, in glomerulonephritis podocytes display some characteristic features of migrating cells. This might explain their ability to break through the parietal epithelium and to become a constituent of early crescents.

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Introduction

Glomerulonephritis is a collective name for a variety of inflammatory diseases of the glomerulus (Jennette and Thomas 2001). Whatever the etiology is, glomerular inflammation always starts in the endocapillary compartment, comprising the capillaries and the mesangium. As long as the disease remains restricted to that compartment spontaneous healing or therapeutic control of inflammation leads to restitution of an almost normal architecture of the glomerulus. It is the encroachment of the disease upon the extracapillary compartment that initiates irreversible injury. The most characteristic extracapillary lesion in glomerulonephritis is the crescent. In crescents the separation between the parietal epithelium and the visceral epithelium is abolished and Bowman's space is locally obliterated. The first stage in the development of a crescent is termed cellular crescent since the cellular components predominate. Cellular crescents are mainly composed of epithelial cells and of macrophages. It is likely that early crescents result from the proliferation of epithelial cells in humans (Boucher et al. 1987; Jennette and Thomas 2001; Magil and Wadsworth 1982; Yoshioka et al. 1987) and in animal models (Cattell and Jamieson 1978; Le Hir et al. 2001; Ophascharoensuk et al. 1998; Oyanagi et al. 2001). Later, probably following rupture of the basement membrane of Bowman's capsule, the crescent becomes infiltrated by macrophages (Boucher et al. 1987; Lan et al. 1992; Oyanagi et al. 2001). Attempts at identification of the epithelial components by immunolabeling mostly revealed the presence of parietal epithelial cells (Boucher et al. 1987; Guettier et al. 1986; Jennette and Hipp 1986; Magil 1985; Nagata et al. 2000; Ophascharoensuk et al. 1998). In the studies cited above variable fractions of cells in the crescent were not labeled, and thus were not positively identifiable. Recently it was found that a large fraction of cells in early crescents in a

classic murine model of anti-glomerular membrane (GBM) glomerulonephritis originate from podocytes (Moeller et al. 2004). This might be due to an incorporation of part of the tuft in the growing crescent. In such a situation podocytes might dedifferentiate and proliferate as seen in collapsing idiopathic glomerulosclerosis and in HIV-associated nephropathy (Barisoni et al. 1999). However, the presence of podocyte bridges in early stages of crescentic glomerulonephritis (Kriz et al. 2003; Le Hir et al. 2001; Neumann et al. 2003) suggests a more active role for podocytes in crescent formation. Before it attaches to the basement membrane of Bowman's capsule, thus forming a bridge, the podocyte has to disrupt the parietal epithelium. This behavior resembles the migration of leukocytes across an endothelial or an epithelial layer since intercellular junctions are not formed between the podocyte and the adjacent parietal epithelial cells (Le Hir et al. 2001). We therefore hypothesized that the acquisition of migratory properties by podocytes might be crucial for the formation of podocyte bridges and of cellular crescents. The following observations in anti-GBM glomerulonephritis in mice support that hypothesis.

Materials and methods

Animals

Female C57BL/6 mice were obtained specified pathogen-free, and kept in sterilized filter-top cages during the experimental period. The experimental protocol was approved by the Cantonal Veterinary Office of Zurich.

Experimental protocol

Mice, 11–13 weeks old, were immunized by subcutaneous injection of 0.2 mg rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA) in 0.2 ml of a 1:1 emulsion with complete Freund's adjuvant (Sigma Chemicals, St. Louis, MO). Six days later (day 0), glomerulonephritis was induced by intravenous injection of 0.4 ml of a 1:10 dilution of rabbit anti-mouse GBM serum (Le Hir et al. 1998), while control animals were not injected after immunization. Urinary protein concentration was evaluated daily from day 3 by use of dipsticks (Albustix; Miles, West Haven, CT). The kidneys were fixed by perfusion 6 days after injection of anti-GBM serum.

Fixation and tissue processing

Mice were anesthetized with 17 mg/kg body weight xylazine hydrochloride and 50 mg/kg body weight ketamine hydrochloride intraperitoneally. Kidneys were fixed by vascular perfusion via the abdominal aorta as described previously (Dawson et al. 1989). The fixative consisted of 3% paraformaldehyde and 0.05% picric acid in 0.06 M cacodylate buffer (pH 7.4; containing 3 mM $MgCl_2$ and adjusted to 300 mosmol with sucrose) and 10% hydroxyethyl starch (HAES; Fresenius, Switzerland). After 5 min the kidneys were washed out by perfusion with 0.1 M cacodylate buffer for 10 min and then removed.

For cryosections coronal kidney slices were mounted with embedding medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands) on cork disks, frozen in liquid propane cooled by liquid N_2, and stored at −80°C until use. For semithin cryosections coronal slices were cut into cubes of 1–2 mm side length and infiltrated with a cryoprotection solution consisting of 2.6 M sucrose with 20% polyvinylpyrrolidone (Sigma) at 4°C for several days before freezing. For paraffin sections kidney halves were dehydrated in graded alcohol series and finally in xylol. They were then embedded in paraffin.

Kidneys used for electron microscopy were not rinsed after perfusion fixation but instead were immersed in the 3% paraformaldehyde fixative solution described above, to which 1% glutaraldehyde was added.

Cell culture

Cultivation of conditionally immortalized mouse podocytes (kindly provided by Dr. P. Mundel, Albert Einstein College of Medicine, Bronx, NY) was done as recently reported (Endlich et al. 2002). In brief, podocytes were maintained in RPMI 1640 medium (Life Technologies, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS; Boehringer Mannheim, Germany), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Life Technologies). To propagate podocytes, cells were cultivated at 33°C (permissive conditions) and the culture medium was supplemented with 10 U/ml mouse recombinant γ-interferon (Life Technologies) to enhance expression of the temperature-sensitive large T antigen. To induce differentiation, podocytes were maintained at 38°C without γ-interferon (non-permissive conditions) for at least 1 week. For immunofluorescence studies, podocytes were seeded on glass coverslips, which were coated with 0.1 mg/ml mouse collagen IV (BD Bioscience, Bedford, MA) for 30 min at room temperature.

Transmission (TEM) and scanning (SEM) electron microscopy

The tissue for TEM was postfixed in 1% OsO_4 and embedded in epoxy resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate. For SEM 2-mm-thick coronal kidney slices were dehydrated through graded acetones, critical-point dried (CPD 030; Baltec CNC- und Ingenieurtechnik, Berlin, Germany) and coated with gold in a sputter coater (Balzers Instruments, Balzers, Liechtenstein).

Immunofluorescence

One-micron-thick semithin cryosections were pretreated with 10% normal goat serum in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). Sections were incubated overnight at 4°C with rat anti-mouse primary antibodies: anti-β1-integrin (clone MB1.2; Chemicon International, Temecula, CA) and anti-ICAM-1 (clone KAT-1; ImmunoKontact, Frankfurt a.M., Germany). Sections were rinsed with PBS and incubated for 1 h at room temperature with Cy3-conjugated goat anti-rat IgG (Jackson), together with fluorescein- or Alexa-phalloidin (Molecular Probes, Eugene, OR) and 4,6-diamidino-2-phenylindole dichloride (DAPI; Sigma). The slides were rinsed with PBS and mounted in Dako-Glycergel (Dako, Carpinteria, CA) containing 2.5% 1,4-diazabicyclo-(2.2.2)-octane (DABCO; Sigma) as a fading retardant, and the sections were examined by epifluorescence microscopy. For β1-integrin and cortactin (primary antibody kindly provided by Dr. X. Zhan, American Red Cross, Rockville, MD) the same procedure was performed on 5-μm-thick cryosections, and the sections were studied by laser scanning microscopy. Cortactin immunoreactivity was also visualized in cultured podocytes as reported earlier (Welsch et al. 2001). Briefly, cells were fixed (2% paraformaldehyde, 4% sucrose), permeabilized (0.3% Triton X-100), and blocked in blocking solution (2% FBS, 2% BSA, 0.2% fish gelatin in PBS) followed by antibody incubation.

Immunohistochemical detection of CD44

Three-micron-thick paraffin sections were deparaffinized and washed in distillated water and PBS. They were blocked with 1.5%

normal rabbit serum for 20 min at room temperature and incubated overnight at 4°C with a rat anti-mouse CD44 antibody (clone L178; Pharmingen, San Diego, CA). Detection with an avidin-biotin-peroxidase kit was performed according to the protocol of the supplier (Vector Laboratories, Burlingame, CA). The sections were then counterstained with the periodic acid-Schiff reagent.

Results

We examined four mice with glomerulonephritis and four control mice on day 6 after induction of the disease. The time-course of histopathological alterations in the present model has been described previously (Le Hir et al. 2001). While podocyte bridges are found already on day 3 they are much more frequent on days 6 and 10. On day 6, in contrast to day 10, the incidence of crescents is still low; it was 8.1±7.4 (SD, n=4) crescents per 100 glomeruli in the animals used in the present study.

Morphology of podocyte bridges

The examination of a large number of podocyte bridges in the electron microscope suggests that the contact of the podocyte with the basement membrane of Bowman's capsule (PBM) is not a static adhesion. The cytoplasmic process extending on the PBM frequently displayed the morphology and the dimensions of a large pseudopod (Fig. 1). Cellular processes of podocytes were often found underneath processes of parietal epithelial cells (Fig. 1) suggesting that podocytes displace parietal epithelial cells while advancing on the PBM. The contact of the podocyte with the tuft was sometimes warranted only by a tiny cytoplasmic process whereas the cell body adhered broadly on the basement membrane of Bowman's capsule (Fig. 2). Bridge-forming podocytes generally displayed less luminal cytoplasmic protrusions (see below) than podocytes which were not involved in bridges. In particular, such protrusions did not appear on cytoplasmic processes which extended on the PBM (Fig. 1).

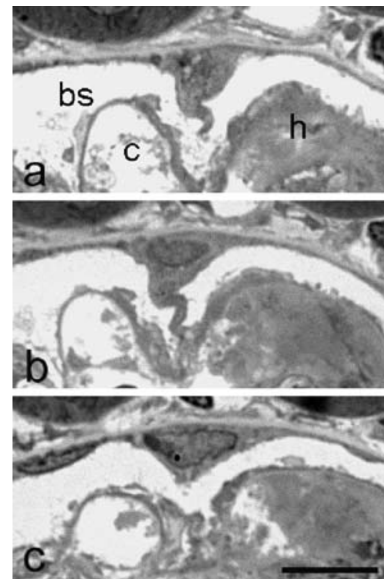


Fig. 2a–c Podocyte bridge in a series of semithin sections. The sections shown in **a**, **b**, and **c** were the number 1, 4, and 8 in a series of sections of about 0.8 μm thickness. *bs* Bowman's space, *c* lumen of necrotic capillary, *h* hyalinized capillary. *Bar* 10 μm

Light and electron microscopy of podocytes not involved in bridges

As described previously (Le Hir et al. 2001) the morphology of podocytes was highly variable in glomerulonephritic animals. Some appeared normal while others showed one or more alterations that have been repeatedly described in different glomerulopathies, namely fusion of foot processes, focal detachment from the basement membrane, vacuolization of the cytoplasm, or formation of microcysts. There was in addition a striking alteration of the luminal membrane. Instead of the almost smooth luminal surface seen in control animals, the majority of podocytes showed numerous microprojections of the cytoplasm into the urinary space (Fig. 3). Two types of microprojections, one slender and the other plump, were identified in SEM and in TEM. Both types were often seen on the same podocyte, but on most podocytes one or the other clearly prevailed. Whereas a few slender microprojections were found in a large proportion of podo-

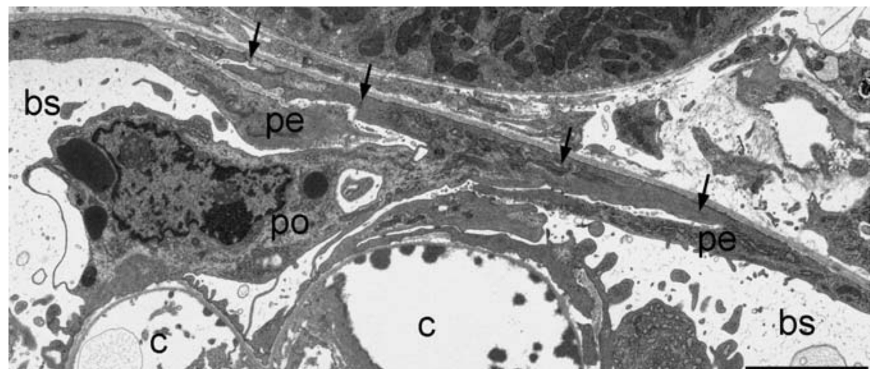


Fig. 1 Podocyte bridge in transmission electron microscopy. A cytoplasmic process (*arrowheads*) of the podocyte displaces parietal epithelial cells from their basement membrane. *bs* Bowman's space, *pe* parietal epithelial cell, *po* podocyte, *c* capillary. *Bar* 5 μm

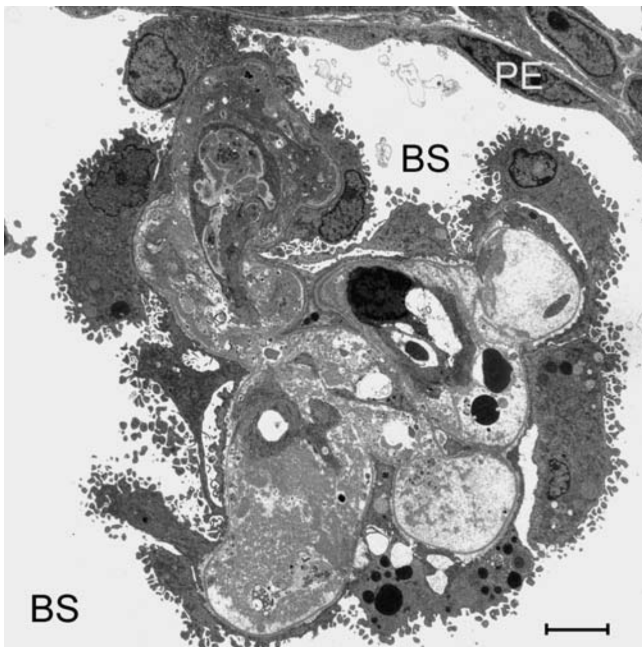


Fig. 3 Transmission electron microscopy of glomerular tuft in glomerulonephritis. Ultrathin section from Epon-embedded tissue. In anti-glomerular membrane (GBM) glomerulonephritis the luminal plasma membrane of podocytes shows many, mainly club-shaped protrusions. *BS* Bowman's space, *PE* parietal epithelial cell. *Bar* 5 μm

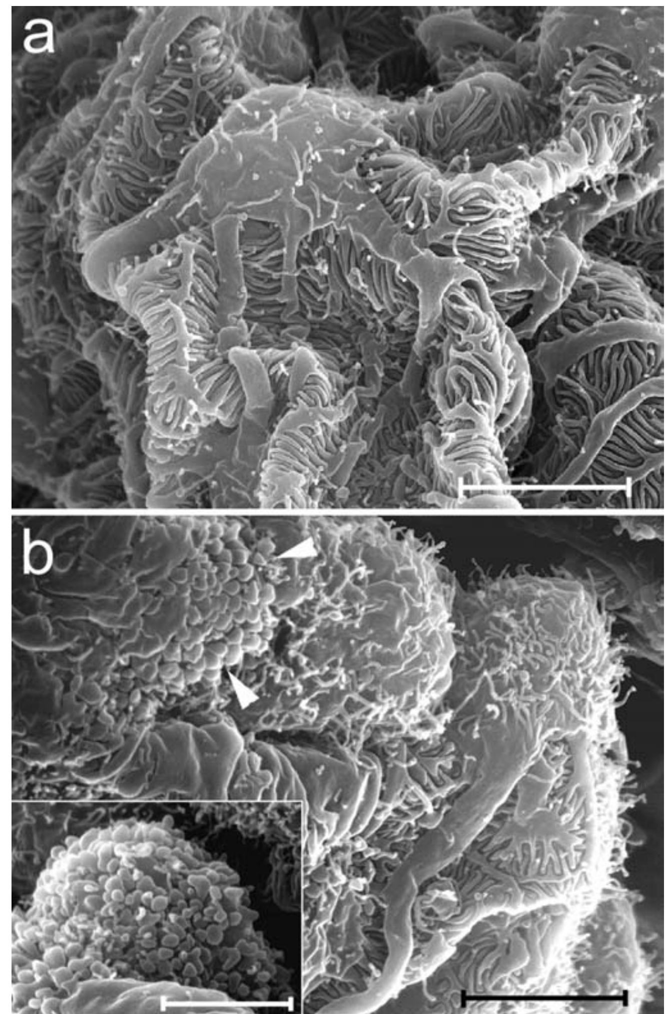


Fig. 4a, b Scanning electron microscopy of podocytes. **a** Podocyte in healthy animal displaying some microvilli-like protrusions on the luminal plasma membrane. **b** In treated animals, podocytes with high incidence of microvilli-like protrusions (*center* and *right*). Other podocytes display club-shaped protrusions all over the cell body (*arrowheads*). In the *inset* microvilli-like protrusions are visible among the club-shaped protrusions. *Bars* 4 μm

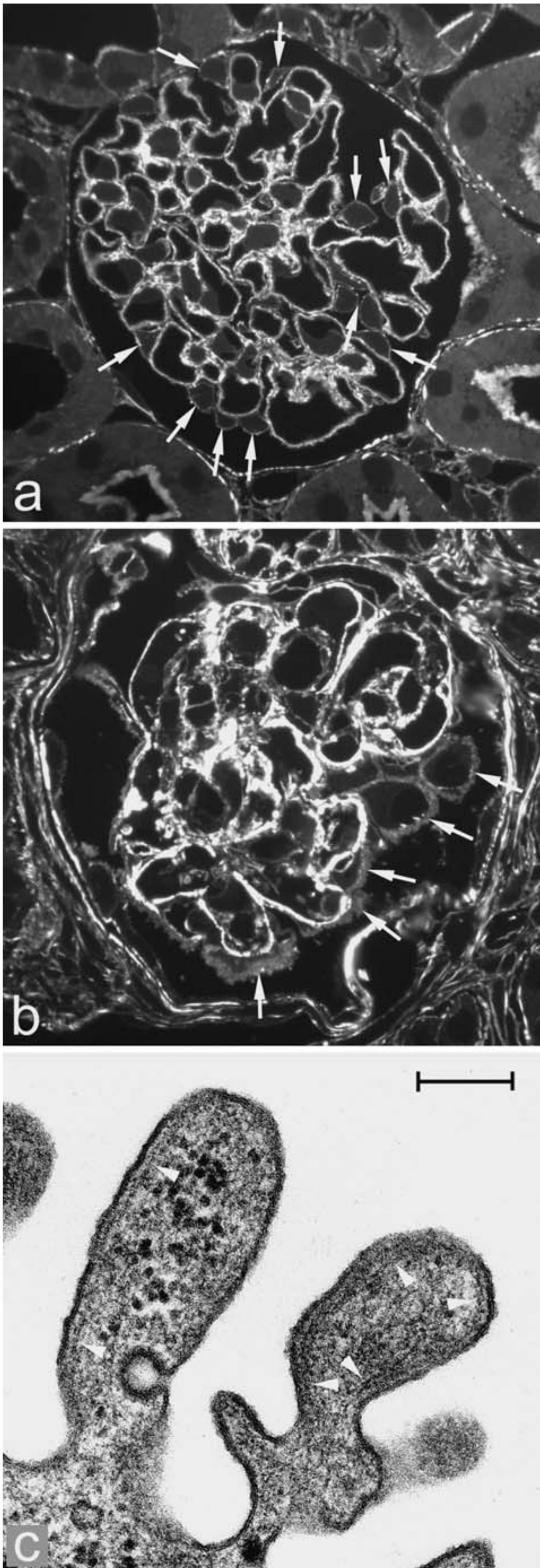
cytes of control mice (Fig. 4a), their incidence increased in glomerulonephritis (Fig. 4b). These cytoplasmic protrusions resembled microvilli and they did not contain any organelles but microfilaments (not shown). Their diameter was evaluated in 24 cross-sections at high magnification in TEM, and it ranged between 70 and 143 nm (mean±SD, 100±22 nm). That variability may be partly due to different diameters of different microprojections but it also reflects changes in diameter along individual microprojections, as seen in SEM. The second type of microprojection was polymorph and plump (Figs. 3, 4), and included organelles such as ribosomes, endoplasmic reticulum, and vesicles, but never mitochondria or Golgi apparatus. Underneath the membrane there was a cortical rim which contained microfilaments (Fig. 5c). The plump microprojections were not seen in control mice.

All alterations described above could be observed already on day 3 after induction of glomerulonephritis. Qualitatively they did not change up to day 10.

Immunohistochemistry

In podocytes of control animals F-actin is mainly localized in the foot processes. Accordingly, labeling with phalloidin-FITC produced a strong linear fluorescence along the GBM. Additionally a thin line of fluorescence marked the cortical cytoskeleton (Fig. 5a). In glomerulonephritis the immunofluorescence became irregular along the GBM. Regions of strong fluorescence probably corresponded to the accumulation of actin filaments in fused foot processes. The thin line of immunofluorescence at the cell cortex was replaced by a broad diffuse border, probably corresponding to the actin cytoskeleton in the cytoplasmic microprojections (Fig. 5b).

In control mice β1-integrin was localized in the foot processes of podocytes but it was absent from their perikaryon (Fig. 6a). In glomerulonephritis roughly half of the podocytes displayed a redistribution of β1-integrin which now was seen also in the perikaryon (Fig. 6b). In semithin (1 μm) cryosections the distribution of β1-integrin in the perikaryon could be analyzed in more detail (Fig. 7). Various levels of immunoreactivity with a punctate pattern were seen in the cytoplasm in glomerulonephritic mice. Additionally in some cells there was a distinct apical localization, probably representing the luminal



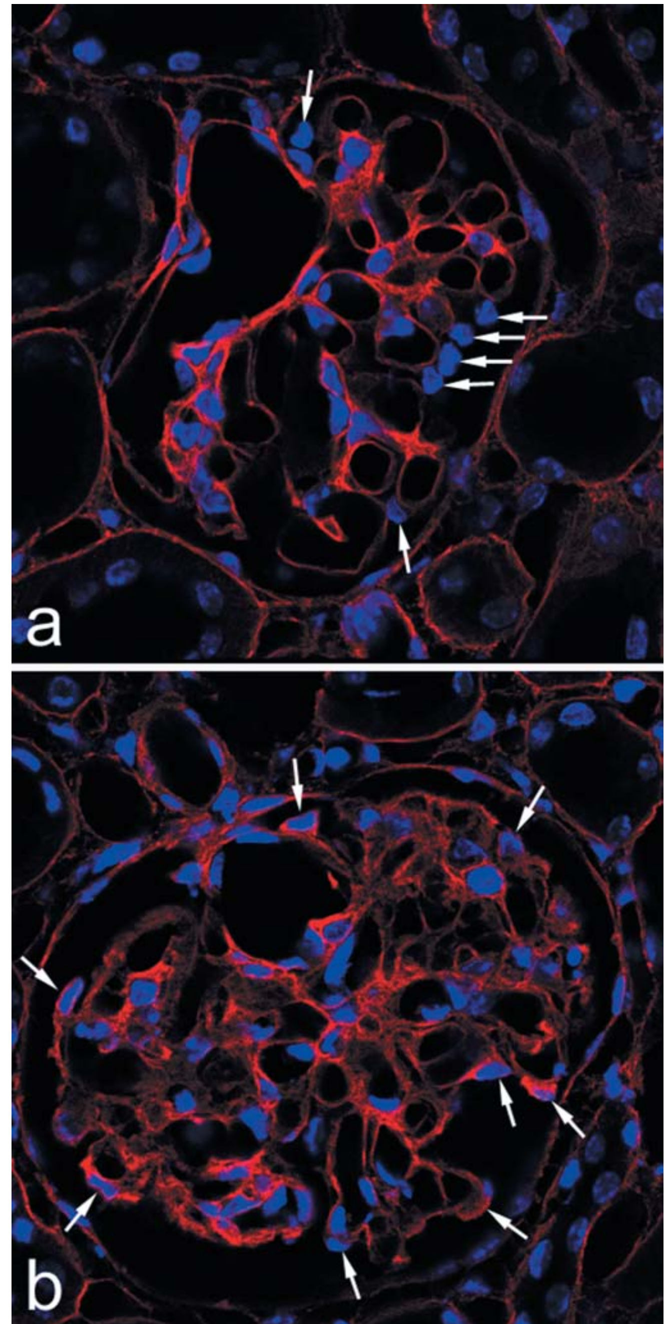


Fig. 6a, b β1-integrin immunoreactivity (red fluorescence). Nuclei display a blue fluorescence (DAPI). **a** In control kidney podocytes (*arrows*) show immunoreactivity for β1-integrin only along the GBM. **b** In glomerulonephritis many podocytes display a strong immunofluorescence signal for β1-integrin also in the cell body

Fig. 5a–c Localization of actin filaments by immunofluorescence and transmission electron microscopy. Fluorescence microscopy on semithin cryosections labeled with phalloidin-FITC (**a**, **b**) and TEM on ultrathin section from Epon-embedded tissue (**c**). **a** In the glomerular overview of control animals immunofluorescence signal with phalloidin shows a thin line along the luminal plasma membrane of podocytes (*arrows*), corresponding to the cortical actin web. **b** In glomerulonephritis the signal for actin is diffuse in many podocytes. **c** Microfilaments (*arrowheads*) are seen under the plasma membrane in club-shaped protrusions. *Bar* 0.2 μm

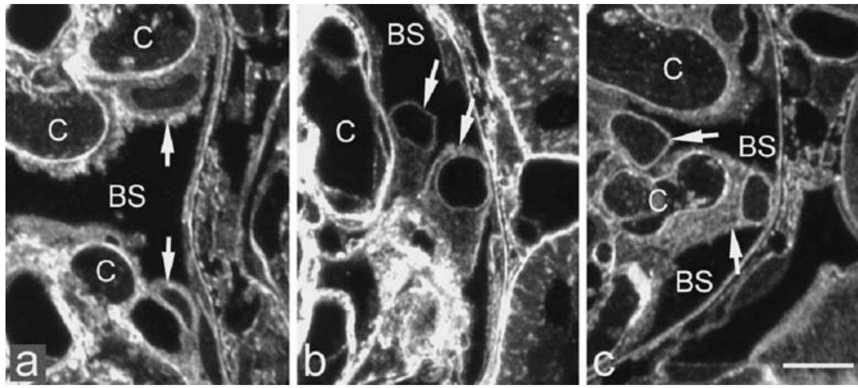


Fig. 7a–c β1-integrin immunoreactivity in glomerulonephritis on semithin cryosections. **a** Podocytes (*arrows*) show an immunofluorescence signal mainly in the luminal plasma membrane protrusions (upper podocyte) and in the perinuclear area (lower podocyte). **b** Perinuclear localization in the two podocytes and in the luminal plasma membrane in the lower podocyte. **c** Particularly strong perinuclear labeling in the upper podocyte, abundant granular cytoplasmic labeling in the lower podocyte. *BS* Bowman's space, *C* capillary. *Bar* 10 μm

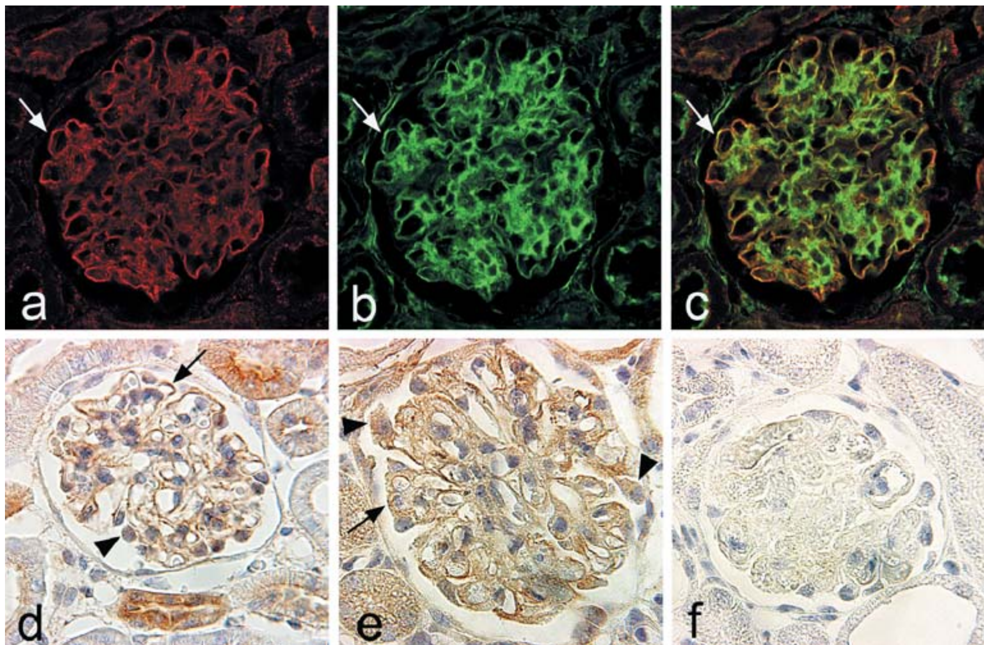


Fig. 8a–f Cortactin immunoreactivity in the glomerulus. Immunofluorescence (**a–c**) and immunoperoxidase (**d–f**). In glomeruli of control mice (**a–c**, **d**) cortactin staining was most intense around capillary loops (*arrows*), where it colocalized with F-actin (cortactin *red* in **a**; F-actin *green* in **b**; images merged in **c**). In glomerulonephritis, cortactin staining was enhanced and its subcellular distribution in podocytes was altered (**e**). Cortactin immunoreactivity around capillary loops, at a site consistent with foot processes, was diffuse (*arrow* in **e** vs **d**). Podocyte cell bodies including the apical cell compartment were also labeled in a diffuse pattern, most likely corresponding to microprojections (*arrowheads* in **e** vs **d**). **f** Negative control without primary antibody. Image width is 110 μm

cell membrane. A perinuclear immunoreactivity was also found in many cells.

Since nothing was known about the expression and distribution of proteins involved in the regulation of actin polymerization in podocytes, we examined the localization of cortactin. In glomeruli of control mice cortactin staining was most intense around capillary loops at a site consistent with localization in foot processes (Fig. 8a, d), where it colocalized with F-actin (Fig. 8b, c). Podocyte cell bodies showed only weak granular immunoreactivity for cortactin. Cortactin was hardly detectable in mesangial cells. In glomeruli of glomerulonephritic animals, overall staining for cortactin was enhanced throughout glomeruli and its subcellular distribution in podocytes was altered (Fig. 8e). Cortactin immunoreactivity around capillary loops, representing foot processes, was no longer prominent. Instead, podocyte cell bodies including the apical cell compartment were labeled in a diffuse pattern, most likely corresponding to labeling of microprojections.

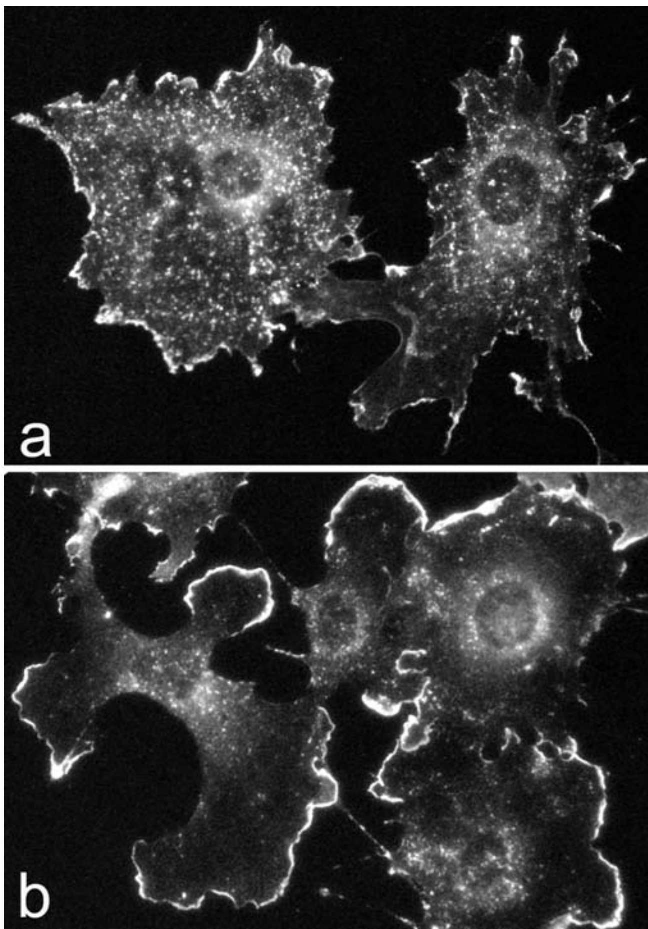


Fig. 9a, b Cortactin redistribution in migrating podocytes. Immunofluorescence on cultured podocytes. In serum-deprived podocytes (2 h in RPMI 1640 without FBS), cortactin labeling was observed at several cell protrusions and throughout the cytoplasm (**a**). In contrast, induction of a migratory phenotype by serum addition (2 h in 10% FBS after 2 h serum deprivation) resulted in formation of lamellipodia and an almost complete redistribution of cortactin to the motile cell margins (**b**). Image width is 130 μm

Since cortactin localizes to dynamic actin structures in various cells, (LaVallee et al. 1998) we examined the localization of cortactin in non-migrating and in migrating cultured podocytes. A non-migrating phenotype was induced by 2 h serum deprivation, and a migrating phenotype was induced by addition of 10% FBS to serum-deprived podocytes. In serum-deprived podocytes cortactin was distributed throughout the cytoplasm, though cell protrusions appeared more intensely labeled (Fig. 9a). Serum addition caused formation of lamellipodia and redistribution of cortactin to the cell margin (Fig. 9b). Thus, cortactin labels sites of actin-driven protrusions in podocytes.

In control mice podocytes were negative for ICAM-1 (Fig. 10a). In glomerulonephritis some podocytes displayed a strong level of immunoreactivity (Fig. 10b). In control mice podocytes were negative for CD44. In glomerulonephritis most of them remained negative. However, a few cells, rarely more than two in a glomerular cross-section, displayed a moderate to strong immunoreactivity (Fig. 11).

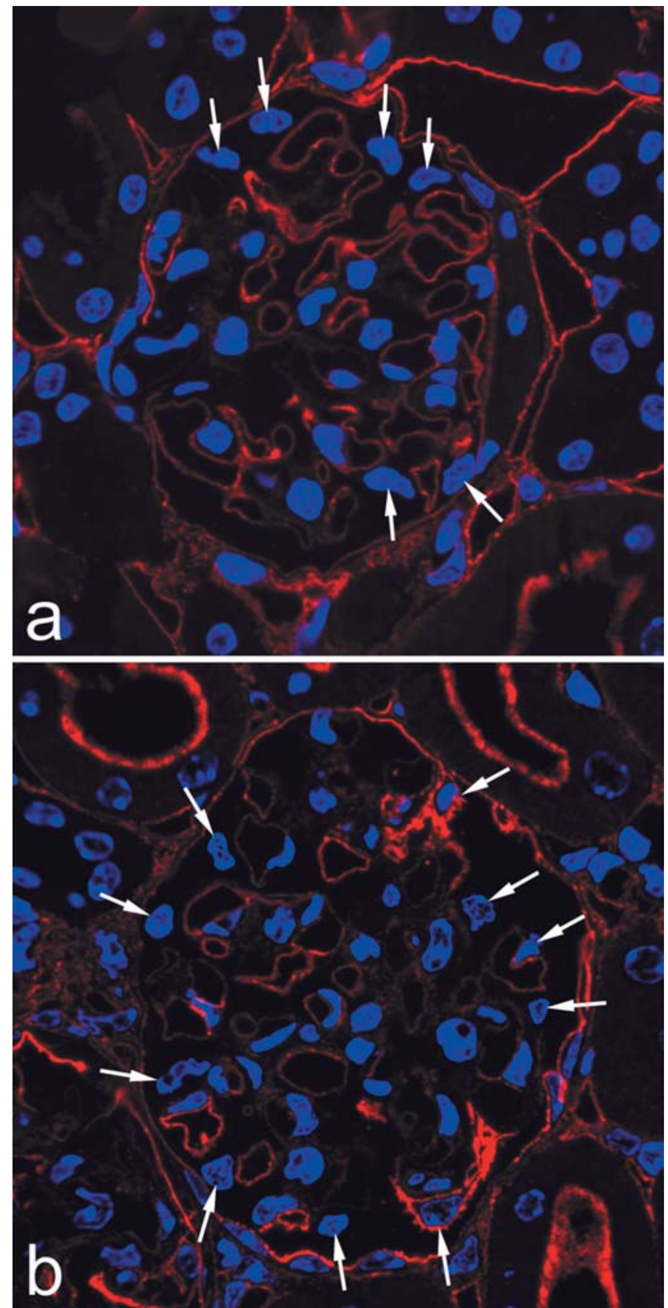


Fig. 10a, b ICAM-1 immunoreactivity (red fluorescence). Nuclei display a blue fluorescence (DAPI). In the glomerular tuft of controls (**a**) only endothelial luminal cell membranes show immunofluorescence signal for ICAM-1. Podocytes (*arrows*) do not show any labeling with anti-ICAM-1 antibody. In glomerulonephritis (**b**) some podocytes are marked for ICAM-1 all over the plasma membrane

Discussion

In a classic model of crescentic glomerulonephritis in mice we previously observed cells that adhered to both the GBM and the PBM, thus building bridges across Bowman's space (Le Hir et al. 2001). They formed foot processes, often displaying slit diaphragms, along the

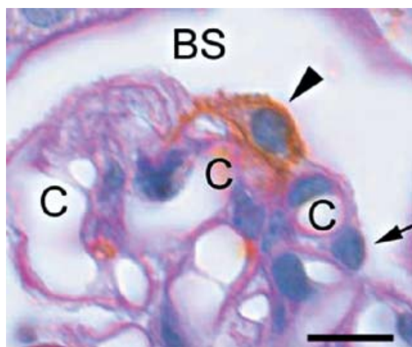


Fig. 11 CD44 immunoreactivity in podocytes in glomerulonephritis. Whereas one podocyte remains unlabeled (*arrow*), the other one displays an intense labeling (*arrowhead*). *BS* Bowman's space, *C* capillary. *Bar* 10 μm

GBM. The cell processes which adhered to the PBM did not form junctional complexes with parietal epithelial cells. The bridging cells displayed several proteins which are expressed by podocytes but not by parietal epithelial cells, namely WT-1, α3 integrin, synaptopodin, podocin, and CD2AP. These features of the bridging cells suggest that they are podocytes and not parietal epithelial cells. In addition, in transgenic mice that expressed β-galactosidase specifically in podocytes, β-galactosidase was expressed by the bridging cells (Moeller et al. 2004). Two mechanisms may be considered, whereby processes of podocytes penetrate the parietal epithelium. One is at work in blastocyst implantation. Penetration of the endometrial epithelium by the trophoblast is brought about by sharing intercellular junctions between the two cell types. In contrast, migrating leukocytes cross epithelia and endothelia by disrupting junctional complexes. The morphology of podocyte bridges suggest that podocytes are engaged in a process which resembles migration of leukocytes. Cytoplasmic processes of podocytes traverse the parietal epithelium without forming junctional complexes. They then extend on large areas of the basement membrane, whereby they displace the parietal epithelial cells (Fig. 1). In some bridges the cell body of the podocyte adheres broadly to Bowman's capsule, while its contact with the tuft is maintained only by one or a few slender processes (Fig. 2).

Not only the formation of podocyte bridges but also the localization of podocytes in crescents, as identified by expression of β-galactosidase in the transgenic model mentioned above, suggests a migratory behavior of these cells in glomerulonephritis (Moeller et al. 2004). The β-galactosidase-positive cells were distributed all over the crescents. They were found mostly close to the PBM and distant from the GBM. Thus, it is likely that, after a podocyte bridge has been formed, the podocyte will eventually detach from the GBM and migrate along the PBM. The occasional observation of bridging cells with a broad adhesion on the PBM and only a residual contact with the GBM (Fig. 2) is suggestive of such a mechanism. Such a behavior of podocytes might be crucial for crescent growth. Indeed, whereas healthy podocytes do not divide, the β-galactosidase-positive, thus podocyte-derived, cells that are found within crescents seem to be dedifferentiated and able to proliferate (Moeller et al. 2004).

Several morphological features of podocytes that are not engaged in bridges were suggestive of a migratory phenotype. The most striking cytological alteration was the formation of cytoplasmic microprojections, often affecting most podocytes in a glomerulus. Some projections resembled microvilli but they showed variability in length and diameter. The other type of microprojection on podocytes was plump and it was not reminiscent of any type of surface differentiation in transporting epithelia. Microprojections on epithelial cells are formed in the context of cell motility (Kijima et al. 2002; Pinco et al. 2002; Small et al. 2002). While it is constitutive in malignant cells, motility is acquired transiently in non-transformed epithelial cells during organogenesis or wound healing (Jacinto et al. 2001). The formation of protrusions in the context of cell motility is driven by the polymerization of actin. The presence of actin in the microprotrusions of podocytes was seen in electron microscopy and by labeling with phalloidin-FITC. The patterns of immunolabeling for cortactin indicate an alteration of the sites of actin polymerization in glomerulonephritis. Cortactin promotes actin polymerization and, accordingly, it has been found in various cell types at subcellular sites where dynamic protrusive structures are formed (Kaksonen et al. 2000; Patel et al. 1998; Sohara et al. 2001; Uruno et al. 2001). In the present study we demonstrated for the first time the presence of cortactin in podocytes in vivo and in vitro. Initiation of migration in podocytes in vitro was accompanied by a redistribution of cortactin to the protrusive cell margin. This shows that cortactin is present at sites of actin polymerization in podocytes. The redistribution of cortactin to the apical cytoplasm of podocytes in glomerulonephritis is thus compatible with the interpretation of the apical protrusions as dynamic structures.

For cell motility protrusive activity has to be linked with adhesion. Thus, induction of cell motility provokes a recycling of β1-integrin from focal contacts to intracellular compartments and then to cell membrane at the leading edge (Andre et al. 1999; Mariotti et al. 2001; Ng et al. 1999; Pierini et al. 2000; Rabinovitz et al. 1999). In human breast carcinoma cells, the transit of β1-integrin through a yet unidentified perinuclear compartment takes place when motility is induced (Ng et al. 1999). In podocytes in glomerulonephritis, we also found a redistribution of β1-integrin from the foot processes to the cytoplasm. Many podocytes showed an accumulation of β1-integrin around the nucleus and at the luminal cell side. Furthermore, we found in some podocytes in glomerulonephritis de novo expression of the adhesion molecules ICAM-1 and CD44 that are also involved in cell motility. Intracellular signaling via ICAM-1 induces PKC activation, Ca^{++} increase, rearrangement of the actin cytoskeleton, and the phosphorylation of cortactin (Adamson et al. 1999; Durieu-Trautmann et al. 1994; Etienne-Manneville et al. 2000). Tyrosine phosphorylation of cortactin is re-

quired for cell migration (Huang et al. 1998). VEGF- and NO-induced migration of endothelial cells in vitro is dependent on ICAM-1 expression (Radisavljevic et al. 2000). ICAM-1 expression by podocytes has already been reported in crescentic glomerulonephritis in the rat (Coers et al. 1994).

As for CD44, its ligation induces outgrowth of lamellipodia (Oliferenko et al. 2000), resolution of intercellular junctions (Cywes and Wessels 2001), and migration (Bourguignon et al. 2001). Ligands of CD44, namely hyaluronic acid and osteopontin, are produced in inflamed glomeruli (Bonvini et al. 2000; Jun et al. 1997; Lan et al. 1998; Sano et al. 2001).

Many factors, which were reported to be upregulated in glomerular inflammation, are capable of inducing cell motility. These factors include heparin-binding epidermal growth factor-like growth factor (HB-EGF; Feng et al. 2000; Polihronis et al. 1996), platelet-derived growth factor (PDGF; Abboud 1995; Eitner et al. 2003), connective tissue growth factor (CTGF; Ito et al. 2001), and transforming growth factor-β (TGF-β; Ito et al. 2001). These growth factors are well known for their potential to induce a migratory phenotype in various cell types through reorganization of the actin cytoskeleton (Matsumoto et al. 1999; Moussad and Brigstock 2000; Raab and Klagsbrun 1997; Zavadil et al. 2001). Furthermore, the expression of CXCR chemokine receptors, which are known to mediate chemotaxis (Bonacchi et al. 2001), is induced in podocytes in membranous glomerulonephritis (Huber et al. 2002).

In the light of the findings in the present study the formation of podocyte bridges might be seen as a migration of podocytes across the parietal epithelial layer, similar to the migration of leukocytes across endothelia and epithelia. If so, and if podocyte bridges represent a step in crescent formation (Kriz et al. 2003; Le Hir et al. 2001; Neumann et al. 2003), an intense protrusive activity in podocytes might be a morphological hallmark of early crescentic glomerulonephritis. The fragmentary data available on the morphology of podocytes in glomerular diseases tend to support that proposal. A high density of plump microprojections (termed “knobs”) together with abundant microvilli appeared to characterize rapidly progressive glomerulonephritis in humans (Jones 1977). Plump microprojections on podocytes have been previously documented in animal models of crescentic glomerulonephritis in mice (Neumann et al. 2003) and in rats (Kriz et al. 2003).

In conclusion, morphological features as well as the expression patterns of various proteins suggest that podocytes in early stages of crescentic glomerulonephritis acquire some properties of migrating cells. By allowing the intrusion of podocytes between the parietal epithelium and its basement membrane the acquisition of a migratory behavior by podocytes might represent a crucial event in the formation of crescents.

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