Pathogenic Old World Hantviruses Infect Renal Glomerular and Tubular Cells and Induce Disassembling of Cell-to-Cell Contacts

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Viral hemorrhagic fevers are characterized by enhanced permeability. One of the most affected target organs of hantavirus-induced hemorrhagic fever with renal syndrome is the kidney, and an infection often results in acute renal failure. To study the underlying cellular effects leading to kidney dysfunction, we infected human renal cell types in vitro that are critical for the barrier functions of the kidney, and we examined kidney biopsy specimens obtained from hantavirus-infected patients. We analyzed the infection and pathogenic effects in tubular epithelial and glomerular endothelial renal cells and in podocytes. Both epithelial and endothelial cells and podocytes were susceptible to hantavirus infection in vitro. The infection disturbed the structure and integrity of cell-to-cell contacts, as demonstrated by redistribution and reduction of the tight junction protein ZO-1 and the decrease in the transepithelial resistance in infected epithelial monolayers. An analysis of renal biopsy specimens from hantavirus-infected patients revealed that the expression and the localization of the tight junction protein ZO-1 were altered compared to renal biopsy specimens from noninfected individuals. Both tubular and glomerular cells were affected by the infection. Furthermore, the decrease in glomerular ZO-1 correlates with disease severity induced by glomerular dysfunction. The finding that different renal cell types are susceptible to hantavirus infection and the fact that infection results in the breakdown of cell-to-cell contacts provide useful insights in hantaviral pathogenesis.
The molecular mechanism, whereby the tubular reabsorption and glomerular filtration function is disturbed, and to what extent both participate in the outcome of the clinical picture of hantavirus infection is not known. Infectious diseases may be associated with a disruption of barrier function due to direct or immune mediated effects on the intercellular integrity. The massive proteinuria observed in hantavirus-infected patients indicates that glomerular and tubular cells are affected (1, 47, 68). However, the susceptibility of human renal cells and the possible effects of hantaviral infection on the barrier function have thus far not been elucidated. In the present study, we examined the infection of human renal cells in vitro and analyzed the integrity of cell-to-cell contacts of infected cells in vitro and in renal biopsy specimens from hantavirus-infected patients hospitalized in our department.

**Materials and Methods**

**Cells and tissues.** Human renal proximal epithelial cells (HREpC) were obtained from Promocell (Heidelberg, Germany) and maintained in renal epithelial cell growth medium 2 (Promocell). Human renal glomerular endothelial cells (HRGEnC) were obtained from Sciencell (Carlsbad, CA) and maintained in endothelial cell medium ECM (Sciencell). Only HREpC and HRGEnC from passages 2 to 6 were used.

The human podocyte cell line was derived from human normal podocytes conditionally transformed with a temperature-sensitive mutant of the simian virus 40 (SV40) large T antigen. Growing at the permissive temperature of 33°C promotes the cell to differentiate. Cells were grown for a period of 14 days at 37°C to ensure differentiation (62). Cells were grown at the permissive temperature of 33°C to inactivate the SV40 T antigen results in a growth arrest and allows the cells to proliferate. Thermoswitching to the nonpermissive temperature of 37°C to 33°C to 37°C and to what extent both participate in the outcome of the clinical picture of hantavirus infection is not known. Infectious diseases may be associated with a disruption of barrier function due to direct or immune mediated effects on the intercellular integrity. The massive proteinuria observed in hantavirus-infected patients indicates that glomerular and tubular cells are affected (1, 47, 68). However, the susceptibility of human renal cells and the possible effects of hantaviral infection on the barrier function have thus far not been elucidated. In the present study, we examined the infection of human renal cells in vitro and analyzed the integrity of cell-to-cell contacts of infected cells in vitro and in renal biopsy specimens from hantavirus-infected patients hospitalized in our department.

**Virus and infection.** The stocks of hantaviral Hantaan virus, strain 76-118 (HTNV) or Puumula virus, strain Vranica (PUUV), were propagated on Vero E6 cells. Virus inocula, HTNV or PUUV, at a multiplicity of infection (MOI) of 0.01 were added to HREpC, HRGEnC, or differentiated podocytes. After incubation for 1 h at 37°C, the unbound virus was removed by a triple washing, and the cells were incubated for the indicated time points at 37°C. The infection was monitored by using immunofluorescence or the Western blot analysis of hantavirus N protein expression with mouse monoclonal anti-nucleocapsid protein (Progen, Heidelberg, Germany) or rabbit polyclonal anti-nucleocapsid protein antibody. An equal loading was verified by the detection of tubulin on the same membrane. For reinfection, Vero E6 cells were inoculated with cell-free supernatants of infected renal cells and monitored for infection for 6 days postinfection (dpi) (HTNV) or 14 dpi (PUUV).

**Immunofluorescence and Western blot analysis.** For immunofluorescence, acetone-fixed cells or frozen sections of renal biopsy specimens were stained with primary and appropriate fluorescently labeled secondary antibodies. The following antibodies were used: mouse or rabbit anti-ZO-1 (Invitrogen, Karlsruhe, Germany), mouse anti-CD31 (Dako, Hamburg, Germany), goat anti-synaptopodin P-19 (Santa Cruz, Heidelberg, Germany), and mouse anti-cytokeratin 18 (Millipore, Schwalbach/Ts, Germany). Integrin was detected with mouse anti-integrin α/β3 (clone LM609; Millipore). To confirm the specificity of anti-integrin α/β3 antibody LM609, fixed cells were incubated with anti-integrin α/β3 antibody that was pretreated with recombinant human integrin α/β3 (R&D Systems, Wiesbaden-Nordenstadt, Germany). Recombinant protein was added to a final concentration of 0.04 μg/μl to integrin α/β3 antibody LM609 (final concentration, 0.01 μg/μl). Images were taken using a Nikon DXM1200C camera attached to a Nikon Eclipse 80i upright microscope (Nikon, Düsseldorf, Germany). The quantification of ZO-1 expression was performed on slides that were all immunolabeled with the mixture of antibodies on the same day. Images on these slides were captured using a constant exposure time. The fluorescence intensity of the selected areas in 32 glomeruli of seven patients and of 18 controls, four samples with normal morphology from nephrectomies were used. Biopsy specimens from hantavirus patients were taken between day 5 and 12 after onset of symptoms. This study was approved by the Ethics Committee of the University Hospital of Heidelberg, and it adhered to the Declaration of Helsinki. Written informed consent was obtained from all patients.

**FIG. 1.** Expression of marker proteins and the hantaviral receptor integrin α/β3 on renal cell types. (A) Human primary cells HREpC, HRGEnC, and human podocytes were stained with antibodies against marker proteins for renal cell types and with anti-integrin α/β3 antibody. (B) Lysates of renal cell types were analyzed for the expression of integrin β3 by Western blot analysis. (C) Flow cytometric analysis of cell surface protein expression of integrin α/β3 and the endothelial marker CD31.
glomeruli of two uninfected control kidneys was measured with Nikon NIS Elements Software. Mean intensities of glomerular ZO-1 staining in renal biopsy specimens of hantavirus patients and controls were statistically compared by using a Student t test. For Western blot analysis, cells were lysed and, after being boiled in sodium dodecyl sulfate (SDS) sample buffer and separated by SDS–10% PAGE, transferred to a nitrocellulose membrane. The protein detection was performed after the incubation with first and peroxidase-conjugated secondary antibodies using the SuperSignal Pico detection kit (Pierce, Bonn, Germany) according to the manufacturer’s instructions. The following antibodies were used: mouse anti-ZO-1 (Invitrogen), mouse anti-β1 tubulin DM 1A (Sigma, Deisenhofen, Germany), and rabbit anti-integrin β3 (H-96; Santa Cruz).

Flow cytometry. For flow cytometry, cells were washed, scraped, and stained with allophycocyanin (APC)-conjugated mouse anti-CD31 antibody (clone AC128; Milteny Biotec, Bergisch Gladbach, Germany) and mouse phycoerythrin (PE)-conjugated anti-integrin αvβ3 antibody (clone LM609). Controls were incubated with APC- and PE-conjugated mouse isotype antibodies. After 1 h, the cells were washed and then analyzed by flow cytometry with FACSCalibur (BD Pharmingen).

Measurement of transepithelial resistance and viability assay. To establish polarized monolayers, HREpC (2 × 10⁵ cells) were plated on 0.4-µm-pore-size 12-well transwell culture system filters (Greiner Bio-One, Frickenhausen, Germany). The integrity of the monolayers was assessed by measuring the transepithelial electrical resistance (TER) with a Millicell-ERS voltmeter (Millipore). At the TER plateau level, cells were infected with HTNV at an MOI of 0.01 or left uninfected, followed by incubation for 1 h at 4°C. Monolayers were washed, and fresh medium was added. The cells were incubated at 37°C, and the TER was monitored at the indicated time points. Uninfected and HTNV-infected HREpC were lysed at day 6 postinfection. The number of viable cells was determined by measuring the amount of ATP using CellTiter-Glo luminescent cell viability assay (Promega, Mannheim, Germany).

RESULTS

Human renal cells express the hantaviral receptor integrin αvβ3 and are susceptible to hantavirus infection. The hantaviral receptor integrin αvβ3 is expressed in endothelial and epithelial cells of different organs and species (18, 65, 69, 70). The Vero E6 cell line of renal tubular epithelial origin, primary
human umbilical vein endothelial cells, or hamster tracheal epithelial cells were used to identify integrin as receptor for Old and New World hantaviruses (20, 22, 43, 59, 61). We analyzed human renal cell types for their susceptibility to hantavirus infection by the detection of the hantaviral receptor integrin \( \alpha_\text{V} \beta_3 \). Primary human renal tubular epithelial cells (HREpC), glomerular endothelial cells (HRGEnC), and podocytes. First, we controlled cells for the expression of cell type-specific marker proteins: HREpC were positive for the epithelial marker protein cytokeratin 18, HRGEnC expressed the endothelial marker CD31, and differentiated podocytes were positive for the podocyte-specific protein synaptopodin. The cell populations showed a homogeneous expression of their respective markers (Fig. 1A). Markers that were not specific for the cell type were not expressed in the cell populations (Fig. 2A). All three renal cell types expressed the receptor for hantavirus infection, as was shown by immunofluorescence analysis for integrin \( \alpha_\text{V} \beta_3 \) (Fig. 1A). To confirm the specificity of the anti-integrin \( \alpha_\text{V} \beta_3 \) antibody LM609, we used a mouse isotype control, along with neutralizing the integrin antibody with recombinant integrin \( \alpha_\text{V} \beta_3 \). In contrast to cells incubated with integrin antibody, cells incubated with isotype control or with preincubated antibody did not show any specific staining (Fig. 2B). The expression of the \( \beta_3 \)-integrin subunit was analyzed by Western blot analysis with a rabbit polyclonal anti-\( \beta_3 \)-integrin antibody. The subunit was detected in all three renal cell types (Fig. 1B). The surface expression of integrin \( \alpha_\text{V} \beta_3 \) on the three renal cell types was analyzed by flow cytometry (Fig. 1C). Human renal glomerular endothelial cells were double positive for the endothelial marker CD31 and for integrin \( \alpha_\text{V} \beta_3 \). In contrast, tubular epithelial cells and podocytes showed surface expression of integrin \( \alpha_\text{V} \beta_3 \), whereas the endothelial marker CD31

FIG. 3. Infection of human renal cells with the hantavirus Hantaan (HTNV). HREpC, HRGEnC, and podocytes were infected with hantavirus HTNV. (A) At the indicated time points, cells were lysed and assessed for expression of viral N protein and tubulin. (B) Cells at 14 days postinfection (dpi) were fixed and immunostained for N protein with anti-N protein and a Cy3-conjugated anti-mouse immunoglobulin secondary antibody. Nuclei were stained with Hoechst 33342. (C) Cells were inoculated with HTNV. At the indicated time points, the cells were fixed and infected cells were quantified by immunostaining for N protein expression. HREpC, gray bars; HRGEnC, white bars; podocytes, black bars. The data are representative of three independent experiments (mean ± the standard deviation [SD]). (D) Cell-free supernatants of uninfected and HTNV-infected renal cells were collected and analyzed for the expression of N protein by Western blot analysis. Vero E6 cells were incubated with supernatants of infected renal cells and stained for hantaviral N protein (red) at 6 dpi.
was absent. Together, results from immunofluorescence staining, Western blot, and flow cytometry demonstrate the expression of integrin in the three different human renal cell types.

**Hantavirus Hantaan infects human tubular and glomerular cells.** In a next step, we analyzed the susceptibility of human renal cells to the HFRS-causing hantavirus Hantaan 76-118 (HTNV 76-118) in vitro. The infection was monitored by the detection of viral N protein by Western blotting (Fig. 3A) and immunofluorescence (Fig. 3B). Infected tubular epithelial and glomerular endothelial cells were detectable by immunofluorescence 2 days postinfection. An infection of podocytes was visible at 4 dpi. The number of infected human renal epithelial cells and podocytes was continuously increasing and after 14 days, ca. 40% of the cells were positive for hantaviral N protein. In contrast, already 4 dpi more than 60% of the human glomerular endothelial cells were infected (Fig. 3C). The identity of infected cell types was confirmed by immunofluorescence analysis of infected cells for specific marker proteins (Fig. 4). The nuclei of uninfected and infected cells appeared intact with no signs of apoptosis (Fig. 3B). To examine the productivity of infection, we analyzed the supernatants (SN) of renal infected cells for the presence of viral N protein by Western blot analysis (Fig. 3D). The release of infectious particles from susceptible renal cells was confirmed by staining the N protein in Vero E6 cells incubated with supernatants of infected renal cells (Fig. 3D). These results demonstrate the productive infection of different renal cell types that contribute to kidney function.

**Hantaviral infection causes the redistribution and decrease of ZO-1.** We examined the impact of a hantavirus infection of renal cells on the integrity of their cell-to-cell contacts by analyzing the localization and the expression levels of ZO-1. The junctional marker protein ZO-1 is present in tight junctions of endothelia, in epithelia, and in the glomerular slit diaphragm of podocytes. In addition to the junctional localization, ZO-1 shuttles between the nucleus and cytosol (4, 7). The localization was examined in infected and uninfected cells. In uninfected cells, the immunostaining displayed the localization of ZO-1 along the contacts of adjacent cells and in the nucleus. In cells infected with hantavirus, ZO-1 exhibited a weaker and discontinuous staining at their margins and a redistribution of the protein to the cytoplasm (Fig. 5A).

To analyze the expression levels of ZO-1 protein in uninfected and infected renal cells, equal amounts of total cell protein were analyzed by Western blotting (Fig. 5B). ZO-1 levels were markedly reduced in hantavirus-infected renal epithelial and endothelial cells and drastically in podocytes. To assess the effects of hantavirus-induced redistribution and reduction of ZO-1 expression on the barrier function, we measured the TER of polarized renal cells. Since podocytes and human renal glomerular endothelial cells in vitro did not form a confluent monolayer with a stable TER, we used renal tubular epithelial monolayers. Primary HREpC were seeded to confluence on transwell filters, and the TERs of HTNV-infected and uninfected polarized monolayers were monitored (Fig. 6A). At early time points after infection, the TERs of infected monolayers were not significantly different from uninfected cells. After 12 h, we observed a decrease in the barrier function of the infected epithelial monolayer that was further reduced to 36.36% ± 2.98% of the TER prior to infection that
FIG. 5. Hantavirus affects localization and expression levels of ZO-1. (A) Uninfected monolayers (upper panels) and monolayers infected with HTNV (lower panels) were stained for the tight junction protein ZO-1 (green) and hantaviral N protein (red) at day 6 postinfection. (B) Uninfected and infected cells were lysed and analyzed for the expression of ZO-1, N protein, and tubulin. Shown is a representative Western blot of three independent experiments.
HANTAVIRUSES DISRUPT RENAL CELL-TO-CELL CONTACTS

Experiments (mean ± the SD). Student t test: * P < 0.05; ** P < 0.01. (B) HREPc were infected with HTNV at an MOI of 0.01, and infection was monitored by the detection of N protein in cell lysates. Viability was assessed 144 h postinfection (hpi) by measuring the amount of ATP. Control cells remained uninfected. Viability of infected cells is presented as a percentage of uninfected cell control values. The data are representative of three independent experiments (mean ± the SD). Student t test, P = 0.818.

Old World hantavirus Puumala infects renal cells and changes the structure of their cell-to-cell contacts. We analyzed whether renal cell types support the productive infection with the pathogenic Old World hantavirus Puumala, which is predominant in Europe and causes a milder form of HFRS called nephropathia epidemica (NE). All three cell types were permissive, as shown by the expression of N protein analyzed by immunofluorescence (Fig. 7A) and Western blot analysis of cell lysates. Infectious particles were produced by all three cell types as demonstrated by the detection of N protein in the supernatant (Fig. 7B) and the reinfection of Vero E6 cells after incubation with supernatants of infected renal cells (Fig. 7C). Furthermore, the alterations of cellular junctions during PUUV infection corresponded to the HTNV-induced changes in ZO-1 localization (Fig. 7D).

Mislocalization and reduced expression of ZO-1 in hantavirus-infected patients. In sections derived from human uninfected adult kidneys, we examined the expression of the receptor for pathogenic hantaviruses, integrin αvβ3. The expression was detected in tubular epithelial cells and in glomerular cells (Fig. 8). Staining of CD31-positive endothelial cells for integrin αvβ3 in peritubular capillaries was not observed. Integrin αvβ3 colocalized with the podocyte marker synaptopodin at the surface of the glomerular basement membrane.

Our data confirm the results of previous immunohistochemistry studies and immunogold analysis that demonstrated the expression of integrin αvβ3 in tubular cells and foot processes of podocytes and the absence from peritubular capillaries (6, 49, 80, 84).

To examine whether the infection with hantavirus leads to an alteration of cell-to-cell contacts in the kidney of hantavirus-infected patients, we examined the localization of the junctional protein ZO-1 in renal biopsy specimens from seven patients suffering from Puumala hantavirus-induced acute renal failure that were treated in our department. Table 1 summarizes patient characteristics. The mean age of the patients was 44.20 ± 12.68 years. The laboratory examinations displayed elevated levels of serum creatinine, lactate dehydrogenase, C-reactive protein, and urea and elevated leukocyte counts. Furthermore, thrombocytopenia and decreased levels of serum albumin were observed in all patients. To analyze the integrity of cell-to-cell contacts in kidneys of hantavirus-infected patients, we performed immunofluorescence stainings for ZO-1 (Fig. 9). The analysis revealed that ZO-1 in tubular epithelial cells of normal control kidneys was expressed in the apical part facing the lumen, indicating that epithelial cells had intact tight junctions. In contrast, tubular ZO-1 in renal biopsy specimens of patients showed an aberrant localization with a rather discontinuous staining at the apical margin and redistribution from the cell-to-cell contacts to the cytoplasm and the nucleus in a diffuse or punctuate pattern. According to these results, tubular tight junctions in patients appeared to be disrupted (Fig. 9A). We analyzed the effects of hantaviral infection on the localization and expression levels of ZO-1 in the glomerular apparatus (Fig. 9B). We observed a weaker staining and a redistribution of ZO-1 in the glomerular tuft. Whereas ZO-1 was concentrated in distinct lines with intense staining along the glomerular capillaries in the control kidneys, the localization in the infected kidneys appeared diffuse cytosolic. A quantification of the mean fluorescence intensity of ZO-1 in the glomeruli revealed a significantly reduced glomerular fluorescence of ZO-1 staining in infected patients (Student t test, P < 0.0001). The mean fluorescence intensity of ZO-1 in the glomerular area of kidneys from infected patients was reduced to 62.33% of uninfected control kidneys (Fig. 9C).

In a next step, we analyzed the correlation of glomerular ZO-1 levels (r = 0.951; P = 0.006). The correlation of these two parameters provides a mechanistic link between the hantavirus-induced effects on cell-to-cell contacts with the glomerular dysfunction leading to proteinuria.

Cell-to-cell contacts are specifically affected in hantavirus-infected patients. The histomorphological picture in renal biopsy specimens of hantavirus-infected patients corresponds...
to acute tubulointerstitial nephritis. However, in contrast to the tubulointerstitial nephritis, the hantavirus infection is characterized by often massive proteinuria, but histopathological glomerular injury is absent or moderate despite proteinuria. Therefore, we compared the structure of tight junctions in biopsy specimens from a patient with non-hantavirus-induced acute tubulointerstitial nephritis and from hantavirus-infected patients. The histopathological analysis revealed the typical tubular injury (edema, tubular dilatation, and inflammatory cell infiltrations) in both diseases (data not shown). A comparison of the localization of the tight junction marker protein ZO-1 revealed that, in contrast to hantavirus infection, the tubular and glomerular junctional structure in the interstitial nephritis showed an intact organization with an uninterrupted belt of ZO-1 in tubules and glomeruli. In contrast, in the kidneys of infected patients, staining of ZO-1 was irregular and faint (Fig. 10). Taken together, the results demonstrate a hantavirus-specific clinical picture with a disruption of junctional structures of tubular and glomerular renal cell types, leading to transient massive proteinuria.
Hantaviral antigen is detected in the glomerular and tubular apparatus. We compared the in vitro susceptibilities of renal cells with the pattern of viral antigen expression in renal cryosections from Puumala-infected patients. Cryosections of hantavirus-seronegative patients served as controls (Fig. 11). Seven biopsy specimens from seropositive patients were analyzed for the presence of hantaviral antigen by immunofluorescence. Hantaviral N protein was detected in podocytes in the glomeruli and in tubular epithelial cells of all infected patients (Fig. 12). The redistribution of ZO-1 occurred in tubules whose cells expressed hantaviral N protein. Whereas ZO-1 in an adjacent uninfected tubule localized at the apical site facing the tubular lumen, staining of ZO-1 in infected tubules displayed a weaker intensity and a more diffused pattern.

DISCUSSION

The epithelium is a critical barrier for pathogens, and entering the target tissue requires strategies to disrupt the integrity of cell-to-cell contacts. The infection of polarized monolayers is often directly linked to the pathogenesis of disease.

The clinical picture of human hantaviral infections differs between Old and New World hantavirus. They may vary in the target organ, in which the infection predominantly manifests. An infection with Old World hantaviruses often leads to acute renal failure with massive proteinuria (1, 51, 71). In recent years reports of cases with pulmonary involvement in Old World hantavirus infection were reported, as well as involvement of other organs (28, 58). The understanding of the susceptibility of human cells for hantavirus infection and the effects of replication on the host cell is crucial for the study of the underlying mechanism of hantaviral pathogenesis. However, the underlying mechanisms of renal dysfunction in HFRS are not yet well understood. The productive infection of renal cells may cause direct effects induced by the viral replication or infection may be responsible for the attack of infected cells by invading immune cells (1, 51, 71). The function of the kidney depends on the glomerular layers (fenestrated endothelium, podocytes, and basement membrane) and the integrity of the tubular epithelium (12, 26, 72). The endothelium of glomerular capillaries differs with its fenestrae from other vascular endothelia that are characterized by tight junction formation, but the fenestrated endothelium works as a molecular filter via its glycocalyx. Podocytes form the barrier via the interdigitated foot processes and their connecting slit diaphragms, specialized multiprotein complexes that share similarities with tight and adherens junctions. A mild dysfunction of the glomerular barrier function can be covered by tubular reabsorption. However, glomerular together with tubular disorder results in proteinuria (11). Our results from cell culture experiments and renal biopsy specimens showed that both systems, glomerular and tubular, show junctional remodeling and may explain the clinical picture of hantavirus-induced acute renal failure that is characterized by massive proteinuria.

Hemorrhagic fever viruses often exert a pronounced tropism for organ-specific epithelia and endothelia. The Nipah virus
infects preferentially endothelial cells of small blood and lymphatic vessels corresponding to the expression pattern of the receptor ephrinB2 (54, 82). The measles virus is able to infect respiratory epithelium, dermal capillary, and microvascular endothelial cells in the brain (2, 83). Studies on dengue virus infection demonstrated viral antigen in endothelial cells of the liver, spleen, and alveoli (31). In vitro studies concerning the pathogenesis of viral hemorrhagic disease often make use of human endothelial cells of the umbilical vein (HUVEC). However, endothelial cells of different organs are heterogeneous, since they are specialized for the function in the respective tissue. They exert a typical morphology and protein expression profile (3, 9, 50, 75). An infection with Old World hantaviruses causes HFRS, and the infection with New World hantaviruses leads to HPS. The fenestrated glomerular endothelium of the kidney differs significantly from the continuous endothelium of other organs (24, 50, 52, 56). The well-defined endothelial specialization and heterogeneity are not only apparent in the tropism of pathogens, many human vascular diseases are limited to distinct types of vessels, e.g., autoimmune diseases that mainly manifest in the kidney (34). The strong association between disease and cell type demands the investigation of the underlying molecular pathomechanism in a cell culture model relating to the relevant target organ (79).

We have shown that the hantavirus receptor integrin $\alpha_\text{v}\beta_5$ is expressed on tubular and glomerular cells of the human kidney and that podocytes and glomerular endothelial and tubular

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*P*, Student $t$ test, one-tailed; $r$, Pearson’s correlation coefficient; CI, 95% confidence interval.

FIG. 9. Alteration in the localization and expression of ZO-1 in kidneys of hantavirus-infected patients. (A and B) Cryosections of renal biopsy specimens of patients infected with hantavirus and of control kidneys were fixed with acetone and stained for the tight junction protein ZO-1. (C) Expression of glomerular ZO-1 was quantified by the measurement of the mean fluorescence intensity in the selected glomerular area (the mean ZO-1 fluorescence intensity of glomeruli of control kidneys was set to 100%).

FIG. 10. ZO-1 localization is not altered in patients with nonhantaviral acute interstitial nephritis. Cryosections of renal biopsy specimens of hantavirus-seronegative patients with interstitial nephritis and hantavirus-infected patients were stained for the tight junction protein ZO-1.
epithelial cells are permissive to infection and release infectious particles. In contrast to the comparable replication kinetics of HTNV in epithelial cells and podocytes, the virus infects the glomerular endothelial monolayer much more efficiently. The infection of the glomerular endothelium may allow the entry of the hantavirus into the kidney with subsequent infection of podocytes and tubular cells. The expression of the hantaviral coreceptor CD55 on different renal cell types may also play a role in the susceptibility (8, 40). An analysis of the impact of infection on the integrity of the cell-to-cell contacts revealed structural alterations in tubular and glomerular cells. We also demonstrated the presence of hantaviral antigen in the kidney and the disruption of junctional structures in biopsy specimens of infected patients. The remodeling affects tight junctions of tubular epithelial cells and the glomerular slit diaphragm between the foot processes of podocytes. Further investigations will focus on possible mechanisms that are either direct, since the viral replication could induce a redistribution of junctional proteins, or the disruption of tight junctions could be a consequence of the effects of cytokines. The induction of the innate immune system by hantavirus infection leads to the secretion of cytokines that may be involved in the signaling cascade controlling epithelial and endothelial permeability (10, 27, 74, 76, 81). A stimulation of hantavirus-infected HUVECs with TNF-α or vascular endothelial growth factor (VEGF) results in a higher permeability than a stimulation of uninfected monolayers (21, 55). VEGF and VEGF receptors 1 and 2 are also expressed in renal cells. Since VEGF plays a crucial role in the maintenance of the filtration barrier (16, 17, 77), the infection with hantavirus could enhance the sensitivity for VEGF in renal cells and increase the permeability the same way as in HUVECs. The role for VEGF in virus-induced renal dysfunction was shown in HIV-associated nephropathy, where the infection of podocytes with HIV induces the expression of VEGF and VEGFR2, leading to podocyte dedifferentiation and disease (38). The histopathological changes in hantavirus infection represent mild tubular interstitial changes and moderate interstitial infiltration of mononuclear cells. However, analyzing the cell-to-cell contacts revealed that the hantavirus-induced acute renal failure differs from interstitial nephritis of nonhantaviral origin that displays no redistribution of junctional proteins.

To summarize, we could demonstrate that renal cells, which are responsible for the function of the kidney, are susceptible to the infection with hantavirus and lose their barrier function by specific remodeling of the structure of cell-to-cell contacts. The disorganization affects both the tubular and the glomerular apparatus, leading to the hantavirus-specific clinical picture that is characterized by renal failure with massive proteinuria.
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