Glomerular endothelium in kidneys with congenital nephrotic syndrome of the Finnish type (NPHS1)

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Abstract

Background. The role of glomerular capillary endothelium in the pathophysiology of nephrotic kidney diseases is poorly known. We analysed the glomerular endothelial lesions in kidneys from patients with congenital nephrotic syndrome of the Finnish type (NPHS1). The disorder is caused by a genetic defect in major podocyte slit diaphragm protein, nephrin. It manifests as nephrotic syndrome soon after birth and leads to glomerular sclerosis in early childhood.

Methods. The glomerular capillary and endothelial cell lesions in NPHS1 kidneys nephrectomized at infancy were studied by electron and light microscopy, immunohistochemistry and cytokine antibody array.

Results. Mesangial expansion and capillary obliteration were evident in practically all NPHS1 glomeruli. No thrombus formation was detected by fibrin staining. Electron microscopy revealed endothelial blebs (endotheliosis). The endothelial fenestration and the attachment of endothelial cells to the basement membrane were, however, quite normal. This fits to the abundant expression of a vascular endothelial growth factor (VEGF) and its transcription factor, hypoxia-inducible factor-1α (HIF-1α), in NPHS1 glomeruli. The proliferative activity of the intracapillary cells was modest and no apoptosis was detected. The expression of an endothelial adhesion molecule, intercellular adhesion molecule 1 (ICAM-1) and several chemokines was upregulated in NPHS1 glomeruli as compared to adult control kidneys. The recruitment of leukocytes carrying ligands for the major endothelial adhesion molecules, however, was modest in the mesangial area of NPHS1 glomeruli.

Conclusions. The findings indicate that the glomerular endothelium is quite resistant to the nephrotic state in NPHS1 kidneys and underscores the importance of mesangial cells in the progression of glomerular sclerosis.

Keywords: glomerular endothelial cells; nephrin; proteinuria

Introduction

Congenital nephrotic syndrome of the Finnish type (NPHS1, CNF) is a rare genetic disorder caused by mutations in the NPHS1 gene [1–3]. This gene encodes for nephrin that is a major structural component of the slit diaphragm connecting podocyte foot processes in the glomerular capillary wall [4]. The podocytes and slit diaphragm are crucial for glomerular sieving, and in NPHS1, the genetic defect in nephrin leads to heavy proteinuria starting already during the fetal period [5]. Nephrotic syndrome with hypoalbuminemia, generalized oedema and hypercholesterolemia starts soon after birth and daily albumin infusions are needed to prevent life-threatening oedema. The Finnish NPHS1 patients are nephrectomized at infancy, and after a short period of dialysis the patients receive a kidney transplant, which is the only curative treatment of this disease [6].

The glomerular epithelial cells (podocytes) in NPHS1 kidneys show extensive foot process effacement that is evident already during the fetal period [5]. After birth, this podocyte damage and nephrosis are associated with a fast progression of glomerular and tubulointerstitial fibrosis [7,8]. Recently, we found that mesangial expansion and obliteration of capillaries were the most significant lesions in NPHS1 glomeruli and probably responsible for the development of glomerulosclerosis [8]. In contrast to the findings in animal models, we did not detect podocyte depletion that would trigger the nephron destruction [9–11].

These findings prompted us to study more thoroughly the glomerular capillaries and endothelial cells in the NPHS1 kidneys. Overall, the importance of glomerular endothelium in the pathophysiology of nephrotic kidney diseases is poorly known. In normal kidneys, the fenestrated endothelium plays a major role in the permeability, vascular tone, coagulation and inflammatory processes within the glomerulus [5]. Endothelial cell injury and dysfunction, on the other hand, are believed to result in cell proliferation and apoptosis as well as swelling and detachment of the endothelial cells from the basement membrane [12]. Conversion of endothelial cell to procoagulant phenotype is reported to lead to fibrin deposition in capillaries, and the
upregulation of endothelial adhesion molecules and the release of cytokines may result in recruitment of leukocytes leading to mesangial lesions [13,14]. In this work, we examined the structural and phenotypic changes of the glomerular endothelium in kidneys nephrectomized at infancy from the NPHS1 patients. These kidneys serve as a unique human material to study the pathology of proteinuric disorders. We were especially interested in the possible signs of endothelial injury and dysfunction that would explain the rapid progression of nephron destruction in NPHS1.

Subjects and methods

Kidney tissue samples

The kidney samples came from 43 children with NPHS1 (age 3–44 months) nephrectomized at the Hospital for Children and Adolescents, University of Helsinki, Finland in 1986–2006. Routine formalin-fixed and paraffin-embedded samples were prepared, and the rest of the renal cortex was snap-frozen in liquid nitrogen and stored at −70°C. All patients had severe nephrotic syndrome from birth and were treated with daily albumin infusions to supplement the continuous heavy protein losses. Since no normal kidneys from age-matched controls were available, we used 10 normal adult kidneys (age 47–58 years) removed for transplantation controls. These kidneys had proved unsuitable for transplantation mainly because of vascular abnormalities. They were snap-frozen and stored at −70°C. In addition, renal biopsy samples from seven patients with tubulointerstitial nephritis or minimal change nephrosis (in remission) were used as controls in transmission electron microscopy (TEM). The age of the patients at the time of biopsies ranged between 1 and 55 years.

Antibodies

Antibodies used for immunofluorescence stainings included anti-fibrin mouse monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-HIF-1α mouse monoclonal antibody (Novus Biologicals, Inc. Littleton, CO, USA), anti-P-selectin goat polyclonal antibody (Santa Cruz Biotechnology, Inc.), and anti-VEGF mouse monoclonal antibody (Santa Cruz Biotechnology, Inc.). Antibodies for immunoperoxidase stainings included mouse monoclonal antibodies against B-cell lymphoma (Bcl)-2 protein (DakoCytomation, Glostrup, Denmark), intracellular adhesion molecule-1 (ICAM-1, CD54) (Novocastra, Newcastle upon Tyne, UK), cyclin A (NCL-cyclin A) (Novocastra), cyclin D1 (NCL-cyclin D1-GM) (Novocastra), endothelial leukocyte adhesion molecule (ELAM-1, E-selectin, CD62E) (R&D Systems, Minneapolis, MN, USA), leukocyte function antigen-1 (LFA-1, CD11a) (Dako, Glostrup, Denmark), MIB-1 (DakoCytomation, Glostrup, Denmark), p53 (DakoCytomation), platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31) (DakoCytomation), P-selectin glycoprotein ligand (PSGL-1, CD162) (Santa Cruz Biotechnology, Inc.), Sialyl-LewisX (sLex, CD15s) (BD Pharmingen, San Jose, CA, USA), vascular cell adhesion molecule-1 (VCAM-1, CD106) (Novocastra), very late antigen-4 (VLA-4, CD49d) (Serotec, Oxford, UK) and von Willebrand factor (vWF) (Santa Cruz Biotechnology, Inc.).

Microscopy and immunohistochemistry

The histological lesions in NPHS1 kidneys were evaluated by light microscopy from paraffin-embedded tissue sections stained with periodic acid silver methenamin (PASM). TEM was performed on (2.5%) glutaraldehyde-fixed samples in a standard fashion [15]. Immunoperoxidase stainings were performed using sections of formalin-fixed, paraffin-embedded or snap-frozen, acetone-fixed cryosections of kidney tissue samples [7,8]. In the immunoperoxidase staining of the cryosections, a peroxidase-conjugated rabbit anti-mouse (Dako, Copenhagen, Denmark) was used as secondary antibody. The reaction was revealed by a 3-amino-9-ethyl carbazole solution containing hydrogen peroxide; Mayer’s hemalum was used for counterstaining. When staining formalin-fixed, paraffin-embedded sections, microwave treatment in 10 mmol/l citric acid for 10 min was performed or Dako Target Retrieval Solution (S1699) (DakoCytomation) was used to improve the antibody penetration. Amplification of the primary antibody reaction was achieved by incubating the sections with biotinylated secondary antibody (Vector Elite ABC Kit) (Vector Laboratories Inc., Burlingame, CA) or TSA Indirect, tyramide signal amplification kit (Perkin Elmer LAS, Inc., NEL700). The TUNEL staining for apoptotic cells was done as described earlier [8]. For the immunofluorescence the cryosections (5 µm) of kidney samples were fixed with acetone or 3.5% paraformaldehyde and stained in a conventional way [16]. Immunostained images and western blot bands were analysed using NIH ImageJ 1.35p program (National Institutes of Health, USA). The program was used to calculate the area fractions of particular immunostained components. The proportion of black-to-white pixels in the image was calculated as percentage. The size of western blot bands was reported as pixels.

Western blotting

The isolation of kidney glomeruli was performed by sieving as described [17]. Isolated glomeruli were homogenized with Ultra-Turrax (Rose Scientific Ltd, Alberta, Canada) in 0.08 M Tris–HCl pH 6.8 buffer containing 2% SDS and 10% glycerol. Western blotting was performed in a conventional way as previously described [7]. Antibodies for western blotting included anti-actin goat polyclonal antibody (Santa Cruz Biotechnology), anti-HIF-1α mouse monoclonal antibody (BD Transduction Laboratories, San Jose, CA), anti-VEGF mouse monoclonal antibody (Santa Cruz Biotechnology), donkey anti-goat IgG-HRP conjugated antibody (Santa Cruz Biotechnology) and goat anti-mouse IgG-HRP conjugated antibody (Santa Cruz Biotechnology).

Cytokine array

Custom human cytokine antibody array (RayBiotech Inc., Atlanta, GA, USA) consisted of 12 different cytokine and
chemokine antibodies spotted in duplicate onto a membrane [7]. The experiments were performed according to manufacturer’s instructions and analysed as previously described [18]. The results were expressed as relative signal intensities so that positive control spots included in each membrane were given an intensity value of 100.

Statistics

Data are presented as mean ± SD when normally distributed or median with IQR otherwise. The differences between control and NPHS1 groups were evaluated using Student’s t-test for continuous variables with normal distribution or otherwise by the Mann–Whitney U-test. P-values < 0.05 were considered significant.

Ethics

The study was approved by the Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki.

Results

The glomerular capillaries in NPHS1 kidneys show obliteration but no thrombus formation

Mesangial expansion with increased amount of cells and matrix, as well as capillary obliteration, was observed in practically all glomeruli in NPHS1 kidneys removed at infancy (Figure 1A, B) [8]. The average diameter of the capillary cross-section in NPHS1 glomeruli with mild and severe mesangial changes was 20% (P < 0.01) and 36% (P < 0.01) shorter than in controls, respectively (134 capillary cross-sections in PASM staining) (Figure 1C). The area fraction of the endothelial marker CD31 showed a 60% (P < 0.01) decrease in NPHS1 glomeruli as compared to controls (Figure 1D–F). On the other hand, no marked fibrin deposition was observed in capillaries of NPHS1 glomeruli (Figure 1G–I). The expression of the pro-coagulant von Willebrand factor (vWF) was also modest on the endothelium (Figure 2A). On average, 5.3 blebs per one capillary cross-section were detected (Table 1). The bleb size varied but no total obstruction of capillary lumen was detected.

The maintenance of the glomerular endothelial cells is largely dependent on vascular endothelial growth factor (VEGF) and its major transcription factor, hypoxia-inducible factor-1α (HIF-1α). VEGF and HIF-1α expression were studied by immunohistochemistry (IHC) and western blotting (WB). The analysis of VEGF expression showed a 1.4-fold (IHC) and 1.8-fold (WB) increase in NPHS1 glomeruli compared to control glomeruli (P-values not significant) (Figure 3A–C and G, H). HIF-1α was upregulated in NPHS1 glomeruli 2.6-fold (P < 0.01) (IHC) and 1.6-fold (P-value not significant) (WB) (Figure 3D–F and G, H). These data are in accordance with the preserved structure of the endothelium.

Little proliferation or apoptosis is detected in the endothelial cells of NPHS1 glomeruli

To evaluate the proliferation state of endothelial cells, immunoperoxidase staining for the cell cycle proteins MIB-1/Ki-67, cyclin D1 and cyclin A was performed (Figure 4A–C). One MIB-1/Ki-67 positive proliferating intracapillary cell was found on average in every other glomerular cross-section in NPHS1 kidneys and in every fifth cross-section in control kidneys (Table 2). The number of positive cells for proliferation markers cyclin A and cyclin D1 were quite similar in NPHS1 and control glomeruli (Table 2). The expression of proapoptotic p53 and anti-apoptotic Bcl-2 was comparable in NPHS1 and control kidneys (Figure 4D, E, Table 2). Apoptosis was not prominent, since no TUNEL-positive intracapillary cells were found in NPHS1 glomeruli (Figure 4F, Table 2).

Endothelial cells and recruitment of leukocytes

To clarify whether vascular endothelium might contribute to the mesangial expansion in the NPHS1 glomeruli, we evaluated the expression of several chemokines, endothelial

Table 1. The electron microscopic findings in NPHS1 and control kidneys

<table>
<thead>
<tr>
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<th>Control</th>
<th>NPHS1</th>
</tr>
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<tbody>
<tr>
<td>No. of glomeruli</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>No. of capillaries</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>No. of visual fields</td>
<td>422</td>
<td>318</td>
</tr>
<tr>
<td>Total length of the GBM (µm)</td>
<td>1012</td>
<td>763</td>
</tr>
<tr>
<td>No. of endothelial filtration pores Per visual field</td>
<td>1.3 (0.5–2.7)</td>
<td>1.2 (0.1–2.6)</td>
</tr>
<tr>
<td>Per 100 µm of GBM</td>
<td>52.6 (34.1–91.7)</td>
<td>48.6 (5.2–89.3)</td>
</tr>
<tr>
<td>No. of blebs/capillaryb</td>
<td>1.0 (0–4)</td>
<td>5.3 (1–14)</td>
</tr>
<tr>
<td>Per capillary cross-section</td>
<td>2.6 (0–20.8)</td>
<td>20.7 (4.6–89.3)</td>
</tr>
</tbody>
</table>

*aThe number of blebs and filtration pores were counted from cross-sections where all the three layers, consisting of endothelium, GBM and foot processes, were seen.
*bMean (range).
*cP < 0.05.
adhesion molecules and their ligands. The glomerular content of 12 major chemokines was analysed by an antibody array. The upregulation of several mediators, such as CCL5 (RANTES), CXCL7 (neutrophil activating peptide 2, NAP-2) and CCL7 (macrophage chemotactic protein-3, MCP-3) was detected (Figure 5). These molecules were upregulated 2.0- to 4.3-fold in NPHS1 glomeruli when compared to control glomeruli.

The expression of endothelial adhesion molecules and their ligands was examined in the glomeruli of control and NPHS1 kidneys by immunohistochemistry stainings. A 4.5-fold ($P < 0.01$) increase in the expression of intercellular adhesion molecule-1 (ICAM-1) was observed in NPHS1 glomeruli (Figure 6A–C). On the other hand, the expression of another important adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1) was not increased (Figure 6D–F). No staining for E-selectin and P-selectin was observed in NPHS1 glomeruli (data not shown). The presence of leukocytes carrying ligands for the major endothelial adhesion molecules was further studied in NPHS1 and control glomeruli. On average, approximately five cells per glomerular cross-section expressed leukocyte function antigen-1 (LFA-1; ligand for ICAM-1) in NPHS1 kidneys. This result indicates that LFA-1 expression was 2.0 ($P < 0.01$) times higher in NPHS1 glomeruli (Figure 6G–I). The number of cells expressing P-selectin ligand (PSGL-1,
Intracapillary expression of cell cycle regulator proteins and TUNEL staining in control and NPHS1 glomeruli

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control (mean ± SD)</th>
<th>NPHS1 (mean ± SD)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>MIB-1</td>
<td>0.20 ± 0.03 (30/150)</td>
<td>0.52 ± 0.03 (149/286)</td>
<td>0.04</td>
</tr>
<tr>
<td>cyclin D1</td>
<td>0.23 ± 0.04 (29/124)</td>
<td>0.21 ± 0.01 (65/303)</td>
<td>NS</td>
</tr>
<tr>
<td>cyclin A</td>
<td>0.00 ± 0.00 (0/150)</td>
<td>0.02 ± 0.003 (6/270)</td>
<td>NS</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.23 ± 0.02 (23/100)</td>
<td>0.17 ± 0.01 (39/227)</td>
<td>NS</td>
</tr>
<tr>
<td>P53</td>
<td>0.14 ± 0.04 (14/100)</td>
<td>0.05 ± 0.006 (12/242)</td>
<td>NS</td>
</tr>
<tr>
<td>TUNEL</td>
<td>0.01 ± 0.00 (1/105)</td>
<td>0.00 ± 0.00 (0/206)</td>
<td>NS</td>
</tr>
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</table>

Abbreviations: Bcl, B-cell lymphoma; MIB, mind bomb homolog; NPHS1, nephritic syndrome of the Finnish type; NS, nonsignificant; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling.

Results are expressed as the mean ± SD of positive cells per glomerular cross-section. In parenthesis: positive cells/glomerular cross-sections examined. Positive intracapillary cells counted only in a high magnification analysis.

Discussion

We evaluated the morphologic and phenotypic changes of glomerular endothelial cells in NPHS1 kidneys that lack the major podocyte slit diaphragm protein, nephrin. In these patients nephrotic syndrome starts after birth, and fast progression of glomerular and tubulointerstitial fibrosis occurs in early childhood. The results in this work demonstrate that glomerular capillaries are involved in the process but the endothelial cells show only few signs of injury.

The overall structure of endothelial cells and their adherence to GBM were quite well preserved in NPHS1 kidneys that fit to the clinical experience that the patients have little haematuria and relatively normal glomerular filtration in spite of the massive protein leakage at the time of nephrectomy. The normal fenestration of endothelial cells, however, was somewhat surprising since the endothelial structure is dependent on the proper function of podocytes, which in NPHS1 undergo severe morphological changes already in the fetal period [7,8,15]. It seems that the structural damage does not impair the functional capacity of podocytes that fit to the normal expression of VEGF in the NPHS1 glomeruli. The endothelial cells and fenestrae are covered by glycocalyx, important for the function of these cells [19,20]. It remains to be solved whether also this layer is intact in NPHS1 glomeruli.

Local lesions in endothelial cells, however, were evident as diffuse oedema and numerous blebs were clearly visible in the capillary cross-sections. This phenomenon, endotheliosis, is a pathogenomic sign of pre-eclampsia, in which the oedematous cells may completely fill the lumen [21–24]. Pre-eclampsia is characterized by proteinuria, hypertension and oedema in late pregnancy and is believed to be caused by circulating molecules (sFLT1, s-endoglin) that inhibit the action of VEGF and transforming growth factor-β (TGF-β). Endotheliosis has rarely been described in proteinuric disorders other than pre-eclampsia [25,26]. Recently, haplosufficient mice for all isoforms of VEGF were shown to develop glomerular endotheliosis underscoring the importance of VEGF for the endothelial integrity [27].

Glomerular endothelial cells are a target of injury in a variety of kidney diseases, such as haemolytic-uraemic syndrome, ischemia, diabetic nephropathy and pre-eclampsia [21,28–30]. The injury may induce remarkable phenotypic changes such as cell proliferation, hypertrophy and apoptosis, regulated by cyclins, cyclin-dependent kinases and their inhibitors [31]. Interestingly, the intracapillary cells in NPHS1 kidneys showed little proliferative or apoptotic activity. Similarly, no upregulation of VWF on endothelial cells, fibrin deposition or thrombi in glomerular capillaries was detected. Such lesions are typical for haemolytic-uraemic syndrome and they result in impaired blood flow and hypoxia in the whole nephron. However, it seems that the NPHS1 glomeruli suffered from some degree of hypoxia since they showed abundant expression of HIF-1α.
Fig. 3. VEGF and its major transcription factor HIF-1α expression was upregulated in NPHS1 glomeruli. (A, B) VEGF expression was studied in four control kidneys (total 70 glomeruli) and five NPHS1 kidneys (total 74 glomeruli) by immunohistochemical stainings. (C) The expression of VEGF was slightly increased (1.4-fold) in NPHS1 glomeruli. (D, E) HIF-1α expression was studied in four control kidneys (total 109 glomeruli) and five NPHS1 kidneys (total 92 glomeruli). (F) A 2.6-fold upregulation in the HIF-1α expression was observed in the glomeruli of NPHS1 kidneys. The difference between control and NPHS1 samples was highly significant. (G) HIF-1α and VEGF expression was detected in isolated glomeruli from three control and three NPHS1 patients using western blotting. Actin staining was used as a loading control. (H) The analysis of HIF-1α and VEGF expression showed a tendency of upregulation in the NPHS1 glomeruli. The data are presented as mean ± SD (magnification ×400, *P < 0.01).

This transcription factor regulates the expression of genes involved in angiogenesis, cell proliferation, apoptosis and energy metabolism, and one of its major functions is to maintain production of VEGF by podocytes [32].

An important sign of endothelial cell injury and dysfunction is the upregulation of leukocyte adhesion proteins on endothelial cell surface [33,34]. This and the synthesis of pro-inflammatory chemokines result in recruitment of circulatory leukocytes that can invade the glomerular mesangium. In NPHS1 glomeruli, the expression of ICAM-1 and several chemoattractants [CCL5 (RANTES), CXCL7 (NAP-2) and CCL7 (MCP-3)] was upregulated as compared to control kidneys. On the other hand, other adhesion molecules, such as VCAM-1, E- and P-selectins, showed little expression on glomerular endothelial cells. VCAM-1 is especially interesting in this context since its upregulation is associated with hypercholesterolaemia and the development of atherosclerosis [35]. As in other nephrotic conditions, the NPHS1 patients have high serum levels of cholesterol and other lipids, which could induce VCAM-1 expression. This was clearly not the case as VCAM1 was abundantly expressed in the Bowman capsule of NPHS1 kidneys, as reported previously in normal kidneys [36], but not in the glomerular tuft.

Mesangial expansion is a constant and early finding in NPHS1 glomeruli and most probably a key factor in the progression of the glomerular sclerosis. It is caused by increased cellularity and increased amount of extracellular matrix [8]. Whether glomerular endothelial cells are involved in this process by recruiting leukocytes is an important question. In this work, we found five LFA-1 and three PSLG-1-positive cells per glomerular cross-section, which were two and six times more than in controls, respectively. On the other hand, the number of VLA-4- and sLEX-positive cells was very low. Overall, the leukocytes represented a very small fraction of the mesangial cellularity. This is in agreement with our previous results showing only few mononuclear and polymorphonuclear cells in the mesangial area [8]. Thus, it seems possible that the heavy protein leakage in NPHS1 kidneys results in mesangial expansion in a similar way as observed in diabetic kidneys with high glucose levels [37].
Fig. 4. The intracapillary expression of cell cycle markers in NPHS1 glomeruli. Endothelial cells in NPHS1 glomeruli showed modest proliferation and no apoptosis. Immunoperoxidase staining of NPHS1 glomeruli samples was carried out with (A) MIB-1, (B) cyclin D1, (C) cyclin A, (D) p53, (E) Bcl-2 antibodies and visualized using light microscopy. Positive intracapillary cells are marked with arrows. (F) TUNEL staining was used to examine apoptosis (magnification ×600).

Fig. 5. Quantification of the glomerular cytokine and chemokine expression. Cytokines and chemokine expression were analysed by cytokine antibody array in six NPHS1 and five control kidneys. The data are presented as mean ± SD (*P = 0.05, **P < 0.01).

A major problem in our studies was that normal adult kidneys and not age-matched kidneys were used as controls. This did not interfere with the structural or cellular analyses of NPHS1 kidneys, but it is clear that especially the results dealing with the expression levels of soluble mediators should be interpreted with caution. Despite this problem, some important conclusions can be drawn: NPHS1 kidneys showed capillary obliteration and signs of glomerular hypoxia. Ultrastructural lesions in endothelial cells were obvious, but the cells showed little proliferative activity or other signs of injury or dysfunction. So, the activation of mesangial cells, not endothelial cell injury, is most probably decisive for the fast development of glomerular sclerosis in NPHS1 kidneys.
Fig. 6. The expression of endothelial adhesion molecules and their ligands in the glomeruli of control and NPHS1 kidneys. The ICAM-1, VCAM-1, LFA-1 and PSGL-1 expression was analysed from four control kidneys and six NPHS1 kidneys. (A, B) ICAM-1 expression was analysed in control (total 112 glomeruli) and NPHS1 (total 118 glomeruli) kidneys by immunohistochemistry. (C) ICAM-1 expression was upregulated 4.5-fold in NPHS1 glomeruli. (D, E) The expression of VCAM-1 in control (total 106 glomeruli) and NPHS1 (total 110 glomeruli) kidneys. (F) VCAM-1 expression was not significantly downregulated in NPHS1 glomeruli. (G, H) LFA-1-positive cells in control (total 134 glomeruli) and NPHS1 (total 121 glomeruli) kidneys are marked with arrows. (I) LFA-1, a ligand for ICAM-1, expression was twice as high in glomeruli of NPHS1 kidneys than in controls. (J, K) PSGL-1 expression in the control (total 116 glomeruli) and NPHS1 (total 120 glomeruli) kidneys (positive cells are marked with arrows). (L) PSGL-1 was upregulated 6-fold in NPHS1 glomeruli. The ICAM-1, VCAM-1, LFA-1 and PSGL-1 expression data are presented as means ± SD (magnification ×400, *P < 0.01).

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Conflict of interest statement. None declared.

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