Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2010;26:311-318

Accepted: July 07, 2010

Uromodulin Facilitates Neutrophil Migration Across Renal Epithelial Monolayers

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Key Words
Uromodulin • Neutrophil • Transepithelial migration • Renal • IgG

Abstract
The glycosylated protein uromodulin is exclusively found in the thick ascending limb cells (TAL) of the kidney, where it is produced on mass and apically targeted, eventually being secreted into the urine. Recently, there has been a renewed interest in this protein due to its ability to interact with the immune system, implicating this protein as a renal inflammatory molecule. Here we investigated the potential role of membrane bound uromodulin on neutrophil adhesion and trans-epithelial migration. The renal tubular epithelial cell line, LLC-PK1, stably transfected with human uromodulin was used to investigate the influence of uromodulin on neutrophil adherence and migration. Uromodulin expression resulted in a significant increase of neutrophil adherence and trans-epithelial migration, in both the apical to basolateral and the basolateral to apical direction. Although uromodulin is GPI anchored and targeted to the apical membrane, we could also observe expression in the basal and lateral membranes domains, which may be responsible for basolateral to apical migration. Furthermore we show that uromodulin binds both the heavy and light chain of IgG, and that IgG enhances neutrophil migration. This study demonstrates that uromodulin can facilitate neutrophil trans-epithelial migration and that this migration can be amplified by co-factors such as IgG.

Introduction

Uromodulin, also known as Tamm-Horsfall protein, is produced exclusively in the thick ascending limb (TAL) of the nephron and is targeted by glycosyl phosphatidylinositol (GPI) to the apical membrane [1]. Uromodulin is abundantly expressed on the external apical membrane of the TAL and is eventually released into the lumen by a poorly characterised process involving proteolysis [2]. Uromodulin is, in healthy individuals, the most abundant urinary protein (excretion rate approx. 50 mg/day) [3, 4], where it is shown to have several beneficial effects including protection against ascending urinary tract infections [5] and prevention of renal stone formation [6, 7].

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Uromodulin has also been shown to interact with components of the immune system generally associated with pro-inflammatory processes [8]. Specifically, uromodulin has been shown to induce pro-inflammatory cytokine release from human whole blood [8], to activate myeloid dendritic cells (DC) to acquire a fully mature DC phenotype [9], and to activate monocytes [8]. Precisely how uromodulin orchestrates these diverse immunological properties is unknown, however the protein has been shown to bind with high affinity to a number of immuno-proteins including the complement factors C1, C1q and C3 [10, 11], IgG [12, 13] and cytokines such as TNF alpha, IL-1 beta and IL-8 [14]. It is thought that these diverse but potent pro-inflammatory properties of uromodulin may signal tubular damage and repair [8, 9].

Neutrophils, the key cells in innate immune response are the first cells arriving at the site of tissue injury or infection and function to eliminate potentially harmful stimuli by the release of antimicrobial proteases, generation of reactive oxygen species and arachidonic acid metabolites [15]. However, to effect these functions they must first migrate from the blood to the site of injury. Neutrophil transmigration involves intricate interaction of neutrophil cell surface molecules with those present on resident endothelial and epithelial cells. There are several main classes of adhesion molecules including, integrins, immunoglobulin gene superfamily (IgSF), selectins and junctional adhesion molecules (JAMs) [16]. The major route of migration across endothelial monolayers is apical to basolateral, but the opposite is true for epithelial cells. Thus, the polarized expression of epithelial/endothelial adhesion molecules is an important factor in controlling the direction of neutrophil transmigration. Neutrophils have been implicated in the progression of many renal diseases [17], including ischemia reperfusion injury [18], diabetic nephropathy [19], interstitial nephritis and chronic nephrotoxicity [20, 21]. In contrast neutrophil function is impaired in chronic renal failure leading to higher susceptibility to infection and sepsis in these patients [17, 22]. Thus the further delineation of pathways involved in neutrophil migration are important for the better understanding of renal physiological and pathophysiological processes.

In this study, using a trans-epithelial neutrophil migration assay previously described [23-25], we investigated the epithelial expression of uromodulin on neutrophil migration. We demonstrate for the first time that uromodulin expression facilitates neutrophil trans-epithelial migration.

Materials and Methods

All chemicals were obtained from Sigma (Vienna, Austria) unless otherwise stated.

Cells and cell culture

LLC-PK1 cells (porcine proximal tubular cell line ATTC no. CL-101) (LLC-PK1^{UMOD-}) and LLC-PK1 cells stably transfected with wild type uromodulin as previously described [4] (LLC-PK1^{UMOD+}) were cultured in DMEM containing 5 mM glucose and 7% FCS with 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C in a humidified 5% CO_2 incubator. LLC-PK1^{UMOD-} cells were used at serial passages from 186 to 220, LLC-PK1^{UMOD+} cells were used at passages from 20 to 45 (where passage was reset to 0 after transfection).

Neutrophils isolation and quantification

Human neutrophils were obtained from peripheral blood of healthy volunteers (anticoagulated with EDTA) by discontinuous density gradient centrifugation on Biocoll Separating Solution (Biochrom, Berlin, Germany), followed by hypotonic lysis of contaminating red blood cells. Cells were resuspended in serum free RPMI-1640 medium containing 0.5% BSA. Cell preparation yielded > 95% neutrophils (by morphology in Giemsa stains) and > 99% viability (by trypan dye exclusion). Neutrophils were quantified by measuring peroxidase activity in 2% Triton-X-100 lysates using the fluorescent Amplex Red assay (Invitrogen, Karlsruhe, Germany). Briefly, 50 µl of whole cell lysates were incubated with 50 µl 8 mM Amplex Red and 1 mM hydrogen peroxide in 96 well plates. After approx. 20 min incubation at room temperature (RT), wells were read at 540 nm excitation and 590 nm emission using a TECAN GENios Plus plate reader. Cell number was calculated by correlation of relative fluorescent units (RFU) values against a dilution of haemocytometer counted neutrophils.

Neutrophil adherence assay

LLC-PK1^{-UMOD} and LLC-PK1^{+UMOD} cells were seeded at 1.0 x 10^5 on 24 well plates and fed every 2 to 3 days until confluence was reached. Isolated neutrophils were added at 1.0 x 10^5 cells per well for 4 h. Monolayers were washed twice in PBS to remove non-adhered neutrophils. Quantification of adhered neutrophils was performed by peroxidase determination in whole cell lysates. In a subset of experiments, neutrophils were pre-labelled for 30 min with 5 µM calcein-AM, for subsequent fluorescent visualization. Images were aquired by an inverted microscope (Axiovert 135 TV) equipped with a 20x (Plan NeoFluar, 0.50 NA) objective and appropriate filter sets for acquisition of calcein fluorescence (ex 490 nm, em 535 nm). To allow for visual comparison, optical settings were kept constant for all images.

Neutrophil Migration studies

LLC-PK1 cells were cultured on 6.5 mm diameter polyester filter inserts with 3 µm pores (Transwell®-Clear, Costar, USA) at a density of 2.0 x 10^5 per insert. For basolateral to apical migration, inserts were inverted for seeding and reverted the following day. Medium was changed every second day. Cells

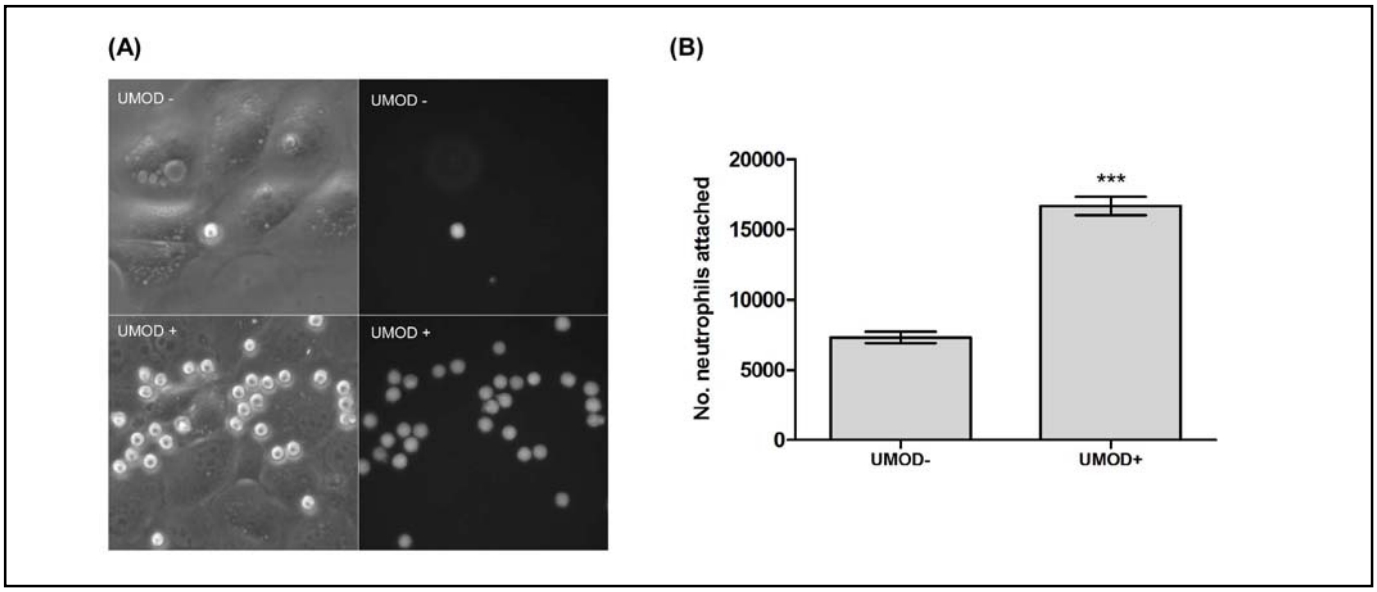

Fig. 1. Effect of uromodulin on neutrophil attachment to LLC-PK1 cells. Non uromodulin expressing (LLC-PK1^{UMOD-}) and uromodulin expressing LLC-PK1 cells (LLC-PK1^{UMOD+}) were cultured to confluence on glass cover slips (A) or 24 well culture dishes (B). Human neutrophils were added to the monolayers for 4 hours and non attached cells were washed away. (A) Phase contrast microscopy (left) and a fluorescent image of calcein-AM pre-labelled neutrophils (right). (B) Quantification of neutrophil attachment using peroxidase determination in whole cell lysates. *** denotes a statistical significance with a P value < 0.001 using an unpaired students t-test.

formed a stable permeability barrier within ~ 5 days (> 95 Ω.cm^2), as assayed by trans-epithelial electrical resistance (TEER), using the Endohm / Evom™ system (World Precision Instruments, Sarasota, USA). Neutrophil migration studies were performed as previously described [25, 26]. Briefly, 2 x 10^5 neutrophils were added to confluent epithelial monolayers for 5 h. The upper chamber was washed twice in RPMI medium and the plates were then centrifuged at 1000 RPM for 10 min. Filters were removed and plates were centrifuged again at 2000 RPM for 10 min. Medium was gently removed and lysis buffer added.

In a subset of experiments epithelial monolayers were pre-incubated with 20 mg/ml IgG or 100 µg/ml LPS for 4 h prior to addition of neutrophils.

Extracellular uromodulin staining

LLC-PK1 cells stably transfected with wild type uromodulin were grown on 25 mm aluminium oxide filter supports with pore size 0.2 µm (Anopore® tissue culture inserts, NUNC, Roskilde, Denmark). After reaching confluence cells were fixed with 2% paraformaldehyde (PFA) in PBS for 5 h at room temperature and over night at 4°C. Then, cells were scraped off. To inhibit the endogenous peroxidases cells were incubated with 0.05% phenylhydrazine in PBS for 1 h at room temperature in the dark. After blocking unspecific binding sites with 10% BSA for 1 h at room temperature cells were incubated in primary goat anti-uromodulin antibody (1/500; MP Biomedicals, Aurora, OH, USA) over night at 4°C followed by the secondary antibody (HRP conjugated rabbit anti-goat IgG, 1/200; Zymed, San Francisco, CA) for 5 h at room temperature (RT). After antibody labeling was completed cells were post fixed in 4% PFA and 0.2% glutaraldehyde for 1 h at RT for superior preservation of ultrastructure. For enzymatic detection of HRP, cells were incubated for 30 minutes in 3,3´diaminobenzidine tetrahydrochloride (DAB) at 0.5% including 0.01% H_2O_2. The reaction was stopped by rinsing in distilled water. Cell monolayers were postfixed for electron microscopy with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, dehydrated in graded series of ethanol and embedded in Polybed (Agar scientific Ltd, Stanstedt, GB). Ultrathin sections were stained by uranyl acetate and lead citrate and analyzed by transmission electron microscopy (TEM) [27]. In negative controls the primary antibodies were omitted and the secondary antibodies were used exclusively. The omission of the primary antibodies resulted in a lack of immunostaining.

Morphology of transmigration

LLC-PK1^{UMOD-} and LLC-PK1^{UMOD+} cells were grown on 25 mm diameter polyester filter inserts with 3 µm diameter pores (Transwell®-Clear, Costar, USA). After reaching confluence cells were washed with PBS, and 1 ml of a 2.0 x 10^5 neutrophil suspension was added to the upper chamber. After 45 min, 1h 30 min and 3 h, cells were fixed in 1% glutaraldehyde in PBS. Cells were washed in PBS, postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, dehydrated in graded series of ethanol and embedded in Polybed (Agar scientific Ltd, Stanstedt, GB). Sectioning for both light (0.5 µm) and electron (0.1 µm) microscopy were performed perpendicular to the cell layer. Sections were stained by toluidine blue for light microscopy or by uranyl acetate and lead citrate for electron microscopy.

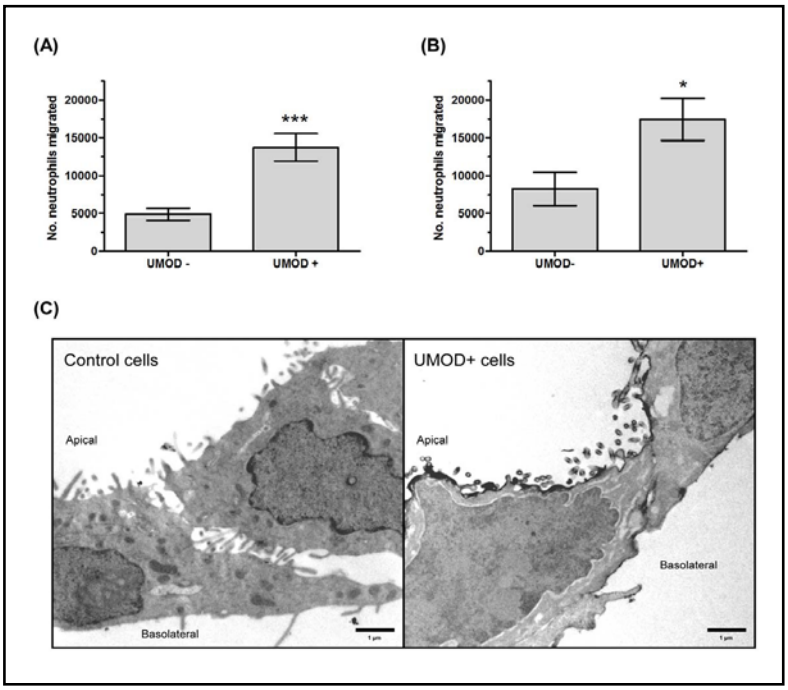

Fig. 2. Uromodulin expression facilitates transepithelial neutrophil migration. Quantification of apical to basolateral (A) and basolateral to apical (B) neutrophil transmigration across LLC-PK1 monolayers. (C) Transmission electron microscopy showing extracellular uromodulin staining in LLC-PK1 $^{UMOD+}$ cells. Un-permeabilised paraformoladehyde fixed cell monolayers were immunostained for uromodulin (see material and methods). Precipitated DAB is electron dense, and thus uromodulin can be seen as the dark staining on the apical, lateral and basal membranes. Non expressing LLC-PK1 cells processed in the same way were used as a control.

Uromodulin binding assay

Human plasma (2.5 µl of a 1 in 20 dilution) and purified human IgG (1 µg) were loaded onto polyacrylamide 4-12 % gradient gels (NuPAGE, Invitrogen). After electrophoresis gels were transferred to Immobilon-P membranes (Millipore, Vienna, Austria) and blocked with 5 % BSA. Membranes were incubated with 1.5 µg/ml purified urinary uromodulin [4]. Uromodulin binding was detected by enhanced chemiluminescence (ECL) after incubation with a HRP conjugated sheep anti-human uromodulin antibody (Biotrend, Cologne, Germany).

Protein determination

Protein was measured with the Bicinchoninic acid (BCA) Protein Assay (Pierce, USA).

Statistical analysis

Statistical differences were tested using an unpaired two-tailed Students t-test or one way analysis of variance with a Dunnett's t-test as indicated.

Results

Uromodulin facilitates neutrophil adhesion to renal epithelial monolayers

Uromodulin expressing cells exhibited a qualitative increase in neutrophil attachment, which appeared to be preferential to the epithelial cell border (Fig. 1A). Quantification of neutrophil attachment using a peroxidase assay, demonstrated that uromodulin expressing cells exhibited a 2.27 ± 0.38 fold increase in neutrophil attachment (Fig. 1B). Although there may seem to be discrepancy between the qualitative and quantitative data it must be pointed out that not all cells in the LLC-PK1 $^{UMOD+}$ population are positive. We have quantified the expression of positive cells in the LLC-PK1 $^{UMOD+}$ population to be 69.5 % ± 3.1 SEM.

Uromodulin expression promotes apical to basolateral and basolateral to apical neutrophil migration

Uromodulin expressing cells also exhibited higher rates of apical to basolateral migration and basolateral to apical migration than non-uromodulin expressing cells (Fig. 2A and B) (2.81 fold ± 1.23 P = 0.0006 and 2.12 fold ± 1.43 P = 0.017 respectively). It was an unexpected finding that uromodulin expression could influence basolateral to apical migration, as uromodulin is GPI anchored and apically expressed. Therefore, we investigated the extracellular expression of uromodulin in LLC-PK1 $^{UMOD+}$ using immunohistochemistry and TEM. Uromodulin was found on the apical pole of the cells as expected with dense staining of the microvilli (Fig. 2C). However, uromodulin was also present in lateral and basal plasma membrane domains.

Light microscopy of neutrophil migration through LLC-PK1^{UMOD+} monolayers show neutrophil adherence, transmigration in processes and migrated neutrophils through intact LLC-PK1 monolayers (Fig. 3A). TEM images show in more detail a neutrophil commencing migration at a cell-cell junction, a neutrophil within the LLC-PK1 monolayer and a migrated neutrophil being followed by a second neutrophil at the same cell to cell junction (Fig. 3B).

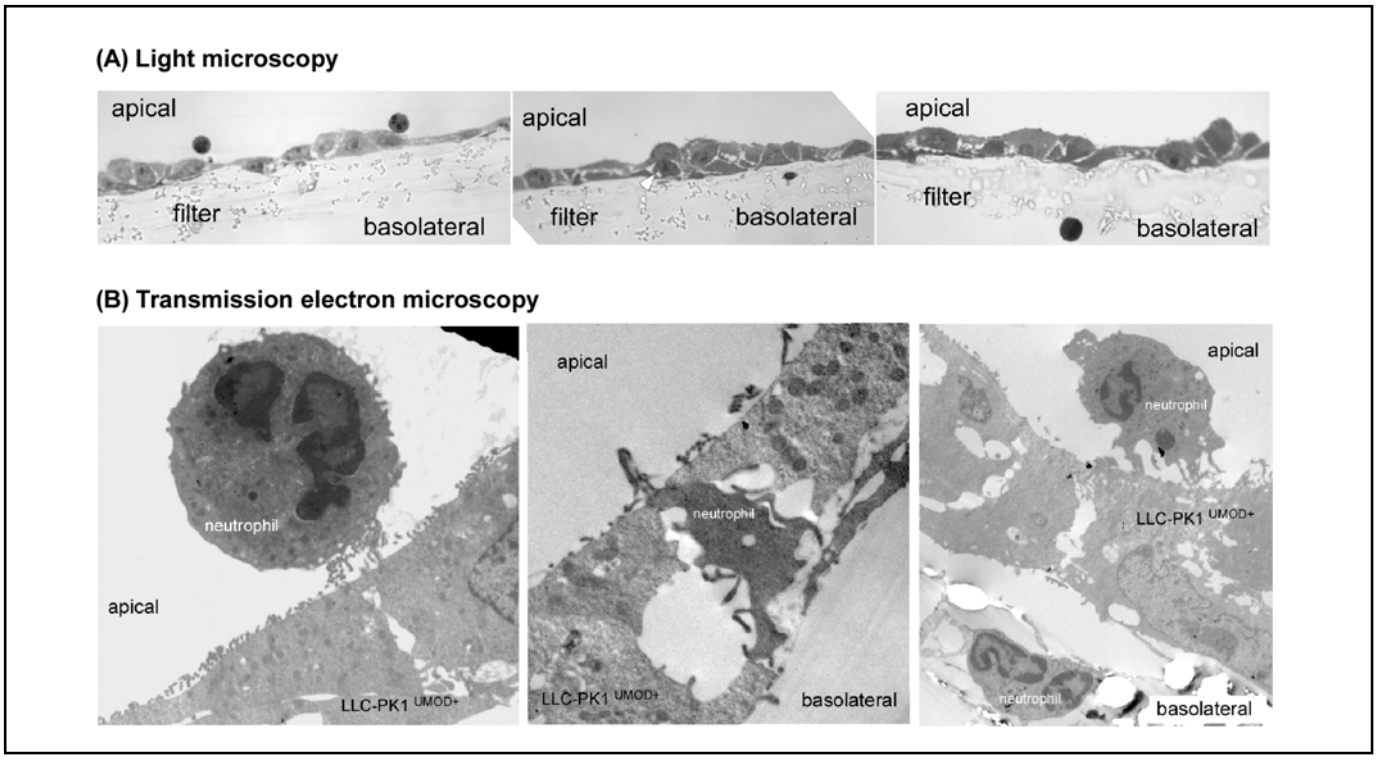

Fig. 3. Light microscopy and transmission electron microscopy of neutrophil migration through uromodulin expressing LLC-PK1 monolayers. (A) Light microscopy of semi-thin sections of neutrophils migrating though LLC-PK1^{UMOD+} cells. The first image shows two neutrophils attaching to the apical surface of the monolayer, the second a neutrophil in the process of migration (white arrow) and finally a fully migrated neutrophil in the filter. (B) Shows TEM of attaching, migrating neutrophils and migrated neutrophils.

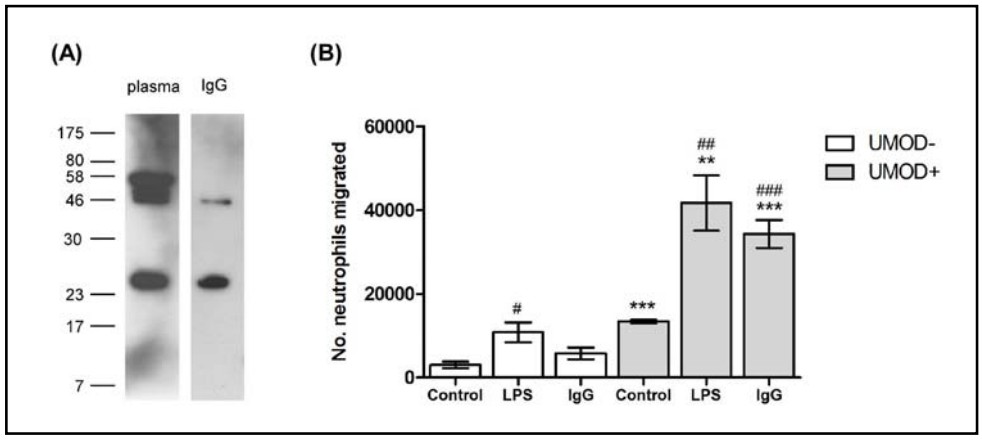

Fig. 4. Effect of LPS and IgG incubation on neutrophil migration through LLC-PK1 monolayers. (A) Human plasma and purified human IgG were ran on SDS-PAGE and immunoblotted with purified human uromodulin. (B) LLC-PK1^{UMOD-} and LLC-PK1^{UMOD+} cells were cultured to confluence on filter inserts. Prior to addition of neutrophils, epithelial monolayers were incubated with either LPS or human IgG. Monolayers were washed and neutrophils were added on the apical side for 5 h. The number of neutrophils migrated was quantified as described. Statistical significance was tested using an unpaired two tailed Student's t-test where, * represents significance versus group control and # represents significance versus treatment with or without uromodulin expression.

Since it has been previously demonstrated that IgG opsonization of HeLa cells expressing uromodulin increases neutrophil adherence [28], we investigated the effect of human IgG on neutrophil migration in our renal epithelial model. Firstly we investigated whether uromodulin is capable of binding human IgG. To this end human plasma and human IgG were separated by SDS-PAGE and transferred to PVDF membranes. Uromodulin bound several proteins in human plasma including the heavy and the light chain of IgG, as revealed by the blot in Fig. 4A. Pre-incubation of LLC-PK1^{UMOD+} cells with 20 mg/ml human purified IgG, increased transmigration by 2.56 ± 0.50 fold over LLC-PK1^{UMOD-} (P = 0.0008; Fig. 4B). (20 mg/ml IgG was used as this is approxi-

mately the upper level in healthy individuals [29]). Thus, IgG bound to uromodulin significantly enhances neutrophil migration. LLC-PK1 monolayers were also pre-incubated with LPS in order to simulate an inflamed tissue as previously described [24, 26]. Uromodulin expressing cells showed a larger LPS response than non expressing cells (4.43 fold ± 1.41, P = 0.0047) (Fig. 4B).

Discussion

Uromodulin is a multifunctional protein primarily involved in renal protection against ascending urinary tract infections, renal stone formation and also in the recruitment of circulating immune cells upon tubular damage. However, these functions are attributed to the secreted form of uromodulin and little attention has been given to possible roles of membrane bound uromodulin under normal physiological conditions and disease states. Many renal diseases involve an immunological component, which is often accompanied by neutrophil infiltration into the tubular interstitium and lumen [30]. Neutrophils are commonly found in the urine and urinary neutrophil presence is increased in renal inflammation and tubular injury [31]. Previous studies have provided some evidence that uromodulin is capable of binding neutrophils, but have not investigated the effect of uromodulin expression on neutrophil trans-epithelial migration [28, 32, 33]. Hence, the aim of this study was to investigate whether membrane bound uromodulin represents a novel renal specific mechanism for facilitation of neutrophil trans-epithelial migration.

For the first time we could demonstrate that polarized renal epithelial cells expressing uromodulin, exhibit both an increased neutrophil adherence and an increased neutrophil trans-epithelial migration. Additionally, uromodulin expression enhanced both apical to basolateral neutrophil migration and basolateral to apical neutrophil migration. Since it is known that uromodulin is a GPI anchored protein [1] and thus targeted to the apical membrane it was initially puzzling why uromodulin expression would result in an enhanced basolateral to apical trans-epithelial migration. One possibility is that uromodulin also acts as a chemoattractant and that neutrophils follow the concentration gradient of secreted uromodulin. Approximately 10 times more uromodulin is secreted into the apical compartment than into the basolateral compartment in uromodulin expressing renal epithelial monolayers [4]. However, a previous study has shown that uromodulin enhances neutrophil response to chemoattractants, but does not itself act as a chemoattractant [30]. Another possibility for uromodulin facilitation of basolateral to apical migration is that, despite GPI anchorage, uromodulin is present in the basolateral membrane. Indeed there is some evidence to support this hypothesis. Several immunohistochemical investigations, including those with human tissue, have shown that uromodulin is also present in the basal and lateral membrane of TAL cells [34-38]. In order to investigate this possibility in our model, we scraped confluent non-permeabilised paraformaldehyde fixed LLC-PK1^{UMOD+} monolayers and conducted pre-embedding immunohistochemistry. This method allows extracellular antibody access to both sides of the monolayer. Electron microscopy revealed a dense apical staining as expected, but also some staining in the lateral and basal membrane (Fig. 2C). Thus, although uromodulin is GPI anchored, a proportion of the protein appears associated to the basolateral domain, possibly by evading the post-translational GPI anchor, as previously proposed [39]. Our observations are in line with the aforementioned tissue staining studies and may also explain the presence of uromodulin in the blood of healthy individuals [4]. Basal and lateral uromodulin deposits are likely to contribute to enhanced basolateral to apical migration of neutrophils due to increased neutrophil adherence.

The potential effects of soluble secreted uromodulin should also be considered. In the cell system utilized, uromodulin secretion is in the range of 100 ng/ml apically and 8 ng/ml basolaterally per 72 h [4]. In the present study epithelial cells were washed prior to neutrophil migration and the migration was conducted for 5 h. Thus the cellular contribution of supernatant uromodulin is 0 at the beginning of the experiments and rises to 7 ng/ml apically and 0.55 ng/ml basolaterally after 5 h. Uromodulin in the serum of normal healthy individuals averages at 13 ng/ml [4, 40], which is more than the contribution of uromodulin secreted by the cells in the 5 h period. Additionally, we have previously demonstrated that uromodulin concentration above 1 µg/ml is necessary to cause IL-8 release from cells in whole blood [40], a finding we have reproduced in isolated neutrophils (not shown). Taken together with the fact that basolateral to apical migration rates of neutrophils was similar to that of apical to basolateral migration rates, it is unlikely that the secreted uromodulin had any major contribution to neutrophil migration in these experiments. However, it is possible and even likely that soluble uromodulin at higher concentrations may play a role in neutrophil migration.

As mentioned, Cavallone et. al. have previously demonstrated that HeLa cells expressing uromodulin require

opsonization [28]. Here opsonization was not necessary for uromodulin enhanced neutrophil attachment or trans-epithelial migration, possibly due to a higher uromodulin expression in our renal epithelial model and thus, an enhanced sensitivity. However, we could demonstrate that incubation with whole human IgG increased neutrophil trans-epithelial migration considerably. Rhodes et. al. originally described the IgG binding potential of uromodulin [12] and Huang et. al. demonstrated that uromodulin binds the light chain of IgG, (binding to the heavy chain was not investigated in that study) [13]. We could show that uromodulin binds both the heavy and the light chains of IgG and this represents a large proportion of uromodulin binding of plasma proteins. This finding is likely to be of clinical relevance in several diseases entities, such as glomerulonephritis or sepsis, where IgG is elevated in the urine [41]. Under these circumstances uromodulin binding with IgG could further enhance neutrophil migration and contribute to renal inflammation. Additionally, we could demonstrate that LPS stimulation of epithelial monolayers caused an enhanced neutrophil migration in uromodulin expressing monolayers. This is possibly due to a LPS induced production of pro-inflammatory factors such as IL-8 [25], and is consistent with a previous observation showing that uromodulin can augment responses to chemoattractants [30].

In conclusion, we could demonstrate that uromodulin expression by polarized renal epithelial cells facilitates both apical to basolateral and basolateral to apical trans-epithelial neutrophil migration. Thus, uromodulin represents a kidney specific neutrophil adhesion molecule, adding another dimension to the role of this protein in the regulation of the innate immune system. Further work will be required in order to investigate the molecular mechanisms of uromodulin neutrophil interaction.

Acknowledgements

This project was funded by the Medizinische Forschungsförderung Innsbruck (MFI) awarded to P.J. and also in part by Oesterrreichische Nationalbank Jubilaeumsfondsprojekt Nr. 12094 (M. J.). We thank Edna Nemati for EM work.

References

1 Rindler MJ, Naik SS, Li N, Hoops TC, Peraldi MN: Uromodulin (tamm-horsfall glycoprotein/uromucoid) is a phosphatidylinositol-linked membrane protein. J Biol Chem 1990;265:20784-20789.

2 Cavallone D, Malagolini N, Serafini-Cessi F: Mechanism of release of urinary tamm-horsfall glycoprotein from the kidney gpi-anchored counterpart. Biochem Biophys Res Commun 2001;280:110-114.

3 Hunt JS, McGiven AR, Groufsky A, Lynn KL, Taylor MC: Affinity-purified antibodies of defined specificity for use in a solid-phase microplate radioimmunoassay of human tamm-horsfall glycoprotein in urine. Biochem J 1985;227:957-963.

4 Jennings P, Aydin S, Kotanko P, Lechner J, Lhotta K, Williams S, Thakker RV, Pfaller W: Membrane targeting and secretion of mutant uromodulin in familial juvenile hyperuricemic nephropathy. J Am Soc Nephrol 2007;18:264-273.

5 Pak J, Pu Y, Zhang ZT, Hasty DL, Wu XR: Tamm-horsfall protein binds to type 1 fimbriated escherichia coli and prevents e. Coli from binding to uroplakin ia and ib receptors. J Biol Chem 2001;276:9924-9930.

6 Hess B, Nakagawa Y, Coe FL: Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. Am J Physiol 1989;257:F99-106.

7 Benkovic J, Furedi-Milhofer H, Hlady V, Cvoriscec D, Stavljenic-Rukavina A: Effect of tamm-horsfall protein on calcium oxalate precipitation. Eur J Clin Chem Clin Biochem 1995;33:705-710.

8 Prajczer S, Heidenreich U, Pfaller W, Kotanko P, Lhotta K, Jennings P: Evidence for a role of uromodulin in chronic kidney disease progression. Nephrol Dial Transplant 2010;25:1896-1903.

9 Saemann MD, Weichhart T, Zeyda M, Staffler G, Schunn M, Stuhlmeier KM, Sobanov Y, Stulnig TM, Akira S, von Gabain A, von Ahsen U, Horl WH, Zlabinger GJ: Tamm-horsfall glycoprotein links innate immune cell activation with adaptive immunity via a toll-like receptor-4-dependent mechanism. J Clin Invest 2005;115:468-475.

10 Rhodes DC: Binding of tamm-horsfall protein to complement 1q and complement 1, including influence of hydrogen-ion concentration. Immunol Cell Biol 2002;80:558-566.

11 Su SJ, Yeh TM: The dynamic responses of pro-inflammatory and anti-inflammatory cytokines of human mononuclear cells induced by uromodulin. Life Sci 1999;65:2581-2590.
12 Rhodes DC, Hinsman EJ, Rhodes JA: Tamm-horsfall glycoprotein binds igg with high affinity. Kidney Int 1993;44:1014-1021.
13 Huang ZQ, Sanders PW: Localization of a single binding site for immunoglobulin light chains on human tamm-horsfall glycoprotein. J Clin Invest 1997;99:732-736.
14 Wu TH, Hsieh SC, Li KJ, Wu CH, Yu CL, Yang AH, Tsai CY: Altered glycosylation of tamm-horsfall glycoprotein derived from renal allograft recipients leads to changes in its biological function. Transpl Immunol 2008;18:237-245.
15 Wright HL, Moots RJ, Bucknall RC, Edwards SW: Neutrophil function in inflammation and inflammatory diseases. Rheumatology (Oxford) 2010, in press.
16 Zemans RL, Colgan SP, Downey GP: Transepithelial migration of neutrophils: Mechanisms and implications for acute lung injury. Am J Respir Cell Mol Biol 2009;40:519-535.
17 Heinzelmann M, Mercer-Jones MA, Passmore JC: Neutrophils and renal failure. Am J Kidney Dis 1999;34:384-399.
18 Awad AS, Rouse M, Huang L, Vergis AL, Reutershan J, Cathro HP, Linden J, Okusa MD: Compartmentalization of neutrophils in the kidney and lung following acute ischemic kidney injury. Kidney Int 2009;75:689-698.
19 Galkina E, Ley K: Leukocyte recruitment and vascular injury in diabetic nephropathy. J Am Soc Nephrol 2006;17:368-377.
20 Kolli VK, Abraham P, Isaac B, Selvakumar D: Neutrophil infiltration and oxidative stress may play a critical role in methotrexate-induced renal damage. Chemotherapy 2009;55:83-90.
21 Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Di Paola R, Britti D, De Sarro A, Pierpaoli S, Caputi A, Masini E, Salvemini D: A role for superoxide in gentamicin-mediated nephropathy in rats. Eur J Pharmacol 2002;450:67-76.
22 Chonchol M: Neutrophil dysfunction and infection risk in end-stage renal disease. Semin Dial 2006;19:291-296.
23 Aydin S, Signorelli S, Lechleitner T, Joannidis M, Pleban C, Perco P, Pfaller W, Jennings P: Influence of microvascular endothelial cells on transcriptional regulation of proximal tubular epithelial cells. Am J Physiol Cell Physiol 2008;294:C543-554.
24 Bijuklic K, Jennings P, Kountchev J, Hasslacher J, Aydin S, Sturn D, Pfaller W, Patsch JR, Joannidis M: Migration of leukocytes across an endothelium-epithelium bilayer as a model of renal interstitial inflammation. Am J Physiol Cell Physiol 2007;293:C486-492.
25 Bijuklic K, Sturn DH, Jennings P, Kountchev J, Pfaller W, Wiedermann CJ, Patsch JR, Joannidis M: Mechanisms of neutrophil transmigration across renal proximal tubular hk-2 cells. Cell Physiol Biochem 2006;17:233-244.
26 Joannidis M, Truebsbach S, Bijuklic K, Schratzberger P, Dunzedorfer S, Wintersteiger S, Lhotta K, Mayer G, Wiedermann CJ: Neutrophil transmigration in renal proximal tubular llc-pk1 cells. Cell Physiol Biochem 2004;14:101-112.
27 Blumer R, Konakci KZ, Pomikal C, Wieczorek G, Lukas JR, Streicher J: Palisade endings: Cholinergic sensory organs or effector organs? Invest Ophthalmol Vis Sci 2009;50:1176-1186.
28 Cavallone D, Malagolini N, Serafini-Cessi F: Binding of human neutrophils to cell-surface anchored tamm-horsfall glycoprotein in tubulointerstitial nephritis. Kidney Int 1999;55:1787-1799.
29 Nieuwenhuys EJ, Out TA: Comparison of normal values of igg subclasses. CollProtides of the biological fluids 1989;36:71-79.
30 Wimmer T, Cohen G, Saemann MD, Horl WH: Effects of tamm-horsfall protein on polymorphonuclear leukocyte function. Nephrol Dial Transplant 2004;19:2192-2197.
31 Simerville JA, Maxted WC, Pahira JJ: Urinalysis: A comprehensive review. Am Fam Physician 2005;71:1153-1162.
32 Kreft B, Jabs WJ, Laskay T, Klinger M, Solbach W, Kumar S, van Zandbergen G: Polarized expression of tamm-horsfall protein by renal tubular epithelial cells activates human granulocytes. Infect Immun 2002;70:2650-2656.
33 Thomas DB, Davies M, Peters JR, Williams JD: Tamm horsfall protein binds to a single class of carbohydrate specific receptors on human neutrophils. Kidney Int 1993;44:423-429.
34 Resnick JS, Sisson S, Vernier RL: Tamm-horsfall protein. Abnormal localization in renal disease. Lab Invest 1978;38:550-555.
35 Zager RA, Cotran RS, Hoyer JR: Pathologic localization of tamm-horsfall protein in interstitial deposits in renal disease. Lab Invest 1978;38:52-57.
36 Fasth A, Hoyer JR, Seiler MW: Renal tubular immune complex formation in mice immunized with tamm-horsfall protein. Am J Pathol 1986;125:555-562.
37 Ishidate T, Ward HJ, Hoyer JR: Quantitative studies of tubular immune complex formation and clearance in rats. Kidney Int 1990;38:1075-1084.
38 Sikri KL, Foster CL, MacHugh N, Marshall RD: Localization of tamm-horsfall glycoprotein in the human kidney using immuno-fluorescence and immuno-electron microscopical techniques. J Anat 1981;132:597-605.
39 Wang J, Shen F, Yan W, Wu M, Ratnam M: Proteolysis of the carboxyl-terminal gpi signal independent of gpi modification as a mechanism for selective protein secretion. Biochemistry 1997;36:14583-14592.
40 Prajczer S, Heidenreich U, Pfaller W, Kotanko P, Lhotta K, Jennings P: Evidence for a role of uromodulin in chronic kidney disease progression. Nephrol Dial Transplant 2010;25:1896-1903.
41 Nasr SH, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, Nadasdy T, D'Agati VD: Proliferative glomerulonephritis with monoclonal igg deposits. J Am Soc Nephrol 2009;20:2055-2064.