Universiteit
Leiden
The Netherlands

Innate immune functions in kidney transplantation

Berger, S.P.

Citation
Berger, S. P. (2009, January 28). *Innate immune functions in kidney transplantation*. Retrieved from https://hdl.handle.net/1887/13439

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/13439

Note: To cite this publication please use the final published version (if applicable).

Chapter 7

Complement activation by tubular cells is mediated by properdin binding

Hilde Gaarkeuken,
Machiel A. Siezenga,
Kim Zuidwijk,
Cees van Kooten,
Ton J. Rabelink,
Mohamed R. Daha and
Stefan P. Berger

Department of Nephrology, Leiden University Medical Center,
Leiden, the Netherlands

Am J Physiol Renal Physiol 2008; 295(5):F1397-403

Summary

Activation of filtered complement products on the brush border of the tubular epithelium is thought to be a key factor underlying proteinuria-induced tubulointerstitial injury. However, the mechanism of tubular complement activation is still unclear. Recent studies on mechanisms of complement activation indicate a key role for properdin in the initiation of alternative pathway. We hypothesized that properdin serves as a focal point for complement activation on the tubulus.

We observed a strong staining for properdin on the luminal surface of the tubules in kidney biopsies from patients with proteinuric renal disease. In vitro experiments revealed dose-dependent binding of properdin to PTEC whereas no significant binding to endothelial cells was detected. Exposure of PTEC with normal human serum as a source of complement resulted in complement activation with deposition of C3 and generation of C5b-9. These effects were virtually absent with properdin deficient serum. Pre-incubation of PTEC with properdin before addition of properdin-depleted serum fully restored complement activation on the cells, strongly suggesting a key role for properdin in the activation of complement at the tubular surface.

In proteinuric renal disease, filtered properdin may bind to PTEC and act as a focal point for alternative pathway activation. We propose that this contribution of properdin is pivotal in tubular complement activation and subsequent damage. Interference with properdin binding to tubular cells may provide an option for the treatment of proteinuric renal disease.

Introduction

Worldwide, the number of patients suffering from chronic kidney disease (CKD) is increasing dramatically [1]. The two most important factors contributing to the global rise in CKD are ageing of the population and the epidemic of type 2 diabetes mellitus [2]. It has been well established now that in chronic kidney disease, regardless of the aetiology, proteinuria is a strong and independent predictor for the progression of chronic renal failure to end-stage renal disease (ESRD) [3;4]. Anti-proteinuric treatment is associated with preservation of renal function [5;6].

Several pathophysiologic mechanisms have been proposed to account for proteinuria-induced tubulointerstitial injury. These include lysosomal rupture due to reabsorbed proteins, oxidative damage induced by transferrin reabsorption, and the stimulatory effects of various plasma proteins on the expression of proinflammatory and profibrotic mediators in renal tubular epithelial cells [7-9]. There is accumulating evidence for complement activation as a powerful mechanism underlying the progression of proteinuric renal disease.

In the setting of proteinuria, plasma complement components may enter the tubular lumen [10]. If these complement components are then locally activated this would lead to cell activation and resulting tubular damage and interstitial fibrosis [11;12]. Indeed, proximal tubular epithelial cells (PTEC) activate serum complement *in vitro* via the alternative pathway [13-15]. Also *in vivo*, both in human chronic proteinuric disease and in experimental models, evidence of complement activation can be detected on the apical surface of the renal tubules [14;16;17]. The protective effect of C6 deficiency in the puromycin model of nephrotic syndrome, as well as in the remnant kidney model, provides further evidence for the role of complement in mediating tubulointerstitial injury [16;18]. Targeting complement inhibitory molecules to the proximal tubules in a rat model of proteinuric kidney disease protects against renal dysfunction [19].

However, the exact mechanism of the unique complement activating property of the proximal tubules has not yet been elucidated. Previous studies reported a role for local ammonium (NH_4) in initiating alternative complement pathway activity [20;21]. We hypothesize that besides ammonium, other mechanisms might be involved in triggering tubular complement activation.

The alternative pathway of complement is triggered by spontaneous hydrolysis of C3, which generates C3a and C3b. Cleavage of C3 results in the formation of a positive feedback loop to produce a rapid local response [22]. Properdin, discovered in 1954

by Pillemer *et al*. [23], is the only known positive regulator of the complement system and consists of dimers, trimers and tetramers arranged in a head-to-tail orientation [24;25]. Properdin binds to C3b and enhances complement activation by stabilizing the alternative pathway C3 convertase [26]. Lately, there has been renewed interest in properdin. It was shown that target-bound properdin may serve as a focal point for amplification of C3 activation. Each subunit in the oligomer provides a ligand-binding site and the unoccupied ligand-binding sites can assemble the alternative pathway convertase on target surfaces [27;28]. It has recently been re-emphasized that properdin may act as a focal point in the activation of the alternative pathway of complement [27-30]. It was suggested already in 1954 that properdin might interact directly with cell surfaces [23;31].
In this study, we show that properdin binds to viable tubular epithelial cells and via this mechanism initiates complement activation.

Materials and Methods

Immunohistochemical staining

Frozen 4 µm tissue sections were used to determine the presence of properdin in cortical tissue of human kidneys. After the sections were fixed with acetone, endogenous peroxidase activity was blocked with 0.1% H_2O_2 and 0.1% NaN_3 for 30 min at room temperature (RT). Then the slides were washed and subsequently blocked with phosphate-buffered saline (PBS), 1% bovine serum albumin (BSA) and 5% heat-inactivated normal human serum for 45 min at RT. Next, sections were incubated with a polyclonal rabbit anti-human properdin antibody (Laboratory of Nephrology, Leiden, the Netherlands) in PBS, 1% BSA and 1% normal human serum in a humid atmosphere overnight at RT. After washing with PBS, antibody binding was detected with horseradish peroxidase (HRP)-labeled goat anti-rabbit Ig (DAKO, Glostrup, Denmark) in PBS, 1% BSA and 1% normal human serum (60 min RT) followed by washing with PBS, incubation with Tyramide-fluorescein isothiocyanate in tyramide buffer (NEN™ Life Science Products, Boston, MA, USA; 20 min RT), washing with PBS, incubation with HRP-conjugated rabbit anti-fluorescein isothiocyanate (DAKO) for 60 min at RT, washing with PBS and development with DAB (Sigma, St Louis, MO, USA). Sections were counterstained with hematoxylin (Merck, Darmstadt, Germany) and mounted with imsol (Klinipath, Duiven, The Netherlands).

Cell culture

The immortalized renal proximal tubular epithelial cell-line HK-2 was kindly provided by M. Ryan, University College Dublin, Ireland [32]. Cells were grown in serum-free DMEM/HAMF12 (Bio-Whittaker, Walkersville, MD) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (Invitrogen, Breda, the Netherlands), insulin (5 µg/ml), transferrin (5 µg/ml), selenium (5 ng/ml), tri-iodothyronine (40 pg/ml), epidermal growth factor (10 ng/ml), hydrocortisone (36 ng/ml, all purchased from Sigma). Primary human proximal tubular epithelial cells (PTEC) were isolated from pre-transplant biopsies or from kidneys not suitable for transplantation and cultured as described earlier [33]. HUVEC were isolated from umbilical cords as described previously [34]. Cells were cultured on a matrix of fibronectin in M199 medium containing 20% heat-inactivated FCS, 100 U/ml penicillin, 100 ug/ml streptomycin, 50 ug/ml Bovine Pituitary Extract (all from Invitrogen) and 10 U/ml heparin (LEO Pharma B.V., Breda, the Netherlands). The cell lines ECRF-24, Jurkat, HL-60 and U937 were cultured as described earlier [35;36].

Isolation of properdin

Properdin was isolated from pooled human donor serum. First, a precipitation step was performed by dialyzing the serum against water containing 5 mM EDTA, pH 6.0. The resulting precipitate was dissolved in Veronal-buffered saline (2x VBS, 1.8 mM Na-5,5-diethylbarbital, 0.2 mM 5,5-diethylbarbituric acid, 145 mM NaCl), dialyzed against 0.01 M NaAc containing 2mM EDTA, pH 6.0 and applied to a Sulphopropyl Sephadex C50 cation exchange colomn (Pharmacia Biotech, Uppsala, Sweden). Properdin was eluted from the column with a linear salt gradient. Properdin-containing fractions, as determined by enzyme-linked immunosorbent assay (ELISA), were pooled, concentrated, and subsequently applied to a Sephacryl S-300 gel filtration column (Pharmacia), after which properdin-containing fractions were pooled. In order to remove contaminating C1q from the preparation, the properdin-pool was dialyzed against PBS, 2 mM EDTA and further purified using human IgG coupled to a Biogel A5 column (Bio-Rad, Hercules, CA). Purity of the properdin preparation was confirmed by analysis on 10% non-reducing SDS-PAGE gel. A single band of 220 kDa was observed.

Serum preparation

Normal human serum was depleted of properdin by immune adsorption using Biogel-coupled anti-human properdin monoclonal antibodies (a gift of State Serum Institute, Copenhagen, Denmark). The properdin-depleted serum showed normal classical and

lectin pathway activity in hemolytic assay. C4-depleted serum, which lacks both classical and lectin pathway activity, was prepared by affinity adsorption using goat anti-human C4 IgG coupled to CNBr-activated Sepharose 4 Fast Flow (Amersham Bioscience Europe, Roosendaal, the Netherlands). After C4 depletion, the serum was free of C4 antigen and classical pathway hemolytic activity could be restored fully by purified hemolytically active C4.

FACS analysis

Deposition of complement on cells was determined by flow cytometry. Properdin binding to the cells was visualized using a polyclonal rabbit anti-human properdin antibody followed by RPE-conjugated goat anti-rabbit IgG (Southern Biotechnology Associates, Birmingham, US). Deposition of C3, C5b-9, C1q, and MBL on the cells was detected using a mouse monoclonal anti-human C3 antibody (RFK22, Laboratory of Nephrology, Leiden, the Netherlands), anti-human C5b-9 (mAb AE11, kindly provided by Dr. T.E. Mollnes, Nordland Central Hospital, Bodo, Norway), anti-human C1q (mAb 2204, kindly provided by Dr. C.E. Hack, Sanquin Research, Amsterdam) and anti-human MBL (mAb 3E7, kindly provided by Dr. T. Fujita, Medical University School of Medicine, Fukushima, Japan) respectively, followed by RPE-conjugated polyclonal goat anti-mouse Ig (DAKO). All antibody incubations were performed on ice for 30 min. Cell surface staining was assessed using a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA). Propidium iodide (1µg/ml, Molecular Probes, Leiden, the Netherlands) was used for exclusion of dead cells.

Properdin binding and complement activation assays

For FACS experiments, cells were grown to confluence in 48-well tissue culture plates. HK-2 cells and HUVEC were exposed to 20% normal human serum diluted in serum-free DMEM/HAMF12 for 2 h at 37°C. C3, C5b-9, C1q, MBL and properdin were assessed on the cell surface by FACS analysis. Alternative pathway mediated complement activation by HK-2 was tested by incubating the cells with 20% normal human serum in the presence of 5 mM Mg EGTA. Properdin binding to HK-2, primary PTEC, HUVEC, ECRF-24, U937, HL-60 and Jurkat was assessed by incubating the cells with purified human properdin (20 µg/ml) diluted in serum-free DMEM/HAMF12 for 1 h at 37°C. Dose-dependent properdin binding to HK-2 and HUVEC was tested by incubating the cells with increasing concentrations of human properdin (10 to 40 µg/ml). The functional consequences of properdin binding were determined by incubating the cells with 5% properdin-depleted, normal human serum or C4-depleted

human serum as a complement source, diluted in serum-free DMEM/HAMF12 culture medium, for 2 h at 37°C after pre-incubation with properdin. Following properdin and/or serum incubation, the cells were washed twice in PBS, harvested by scraping and resuspended in FACS-buffer (1% BSA and 0.02% sodium azide in PBS) for FACS staining.

Results

Properdin is present on the tubular brush border in proteinuric kidneys

The presence of properdin on the brush border of the proximal tubules was determined in renal biopsies of three patients with membranous nephropathy and in pretransplant renal biopsies of three living related kidney donors. Properdin could be detected along the brush border of the tubules in diseased kidneys, whereas properdin was absent in the tubules of healthy kidney tissue (Figure 1). Since the presence of properdin on the tubular brush border of proteinuric kidneys does not distinguish where in the cascade of complement activation properdin comes in, we proceeded to *in vitro* studies to determine whether properdin is an initiating factor in tubular complement activation.

Complement activation by HK-2 cells

Incubation of Human Kidney-2 (HK-2) cells with normal human serum resulted in fixation of complement products on the cell surface. C3, C5b-9 and properdin, but not C1q and mannan-binding lectin (MBL) could be detected (Figure 2a). The complement system was activated on the cell surface via the alternative pathway since deposition of C3 and C5b-9 was unaffected by Mg EGTA, which interferes with the classical and lectin pathway of complement by chelating calcium (Figure 2b). In contrast, complement fixation was completely blocked by EDTA, which inhibits all three pathways of complement activation. C3 and C5b-9 deposition was also detected on HK-2 cells after exposure to C4-depleted human serum, which excludes involvement of the classical or lectin pathway (Figure 2c). To assure that complement activation was localized to the apical surface, serum incubations were performed on cells that were grown to confluence in a tissue culture plate. Human umbilical vein endothelial cells (HUVEC) were used as a control. No complement deposition was observed on these cells after treatment with normal human serum.

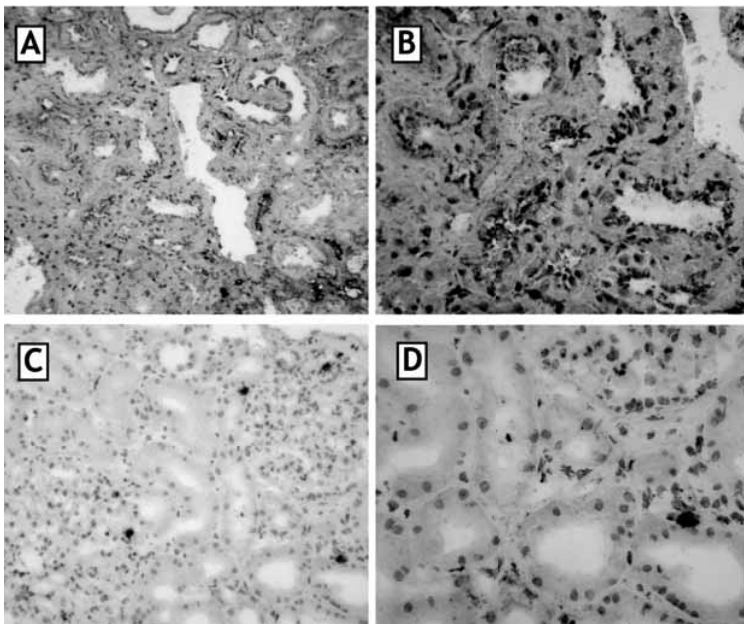

Figure 1. **Properdin staining on the tubular brush border in proteinuric kidneys.** Cryosections of (**A** and **B**) a renal biopsy of a patient with membranous nephropathy and (**C** and **D**) a pretransplant biopsy of a healthy donor were stained immunohistochemically for properdin. (**A** and **C**) Original magnifications were either × 100 or (**B** and **D**) × 250. Pictures are representative for three patients with membranous nephropathy and three healthy kidneys donors (see page 159 for color image).

Binding of properdin to HK-2 cells

We then questioned whether properdin could bind to tubular cells prior to the activation of complement and the deposition of its known ligand C3b. In order to study binding of properdin to the cell surface, confluent cells in a tissue culture plate were incubated with purified human properdin at a concentration of 20 µg/ml. Properdin binding was analysed by flow cytometry. Only cells which were negative for propidium iodide staining were analysed in order to exclude properdin binding to dead cells. As shown in Figure 3a, strong binding of properdin to viable HK-2 cells was detected, whereas no significant binding was shown on HUVEC. As a negative control, the fluorescence intensity of cells incubated with detection antibody only, i.e., without pre-incubation with properdin, is shown. Properdin binds to viable HK-2 cells in a dose-dependent manner (Figure 3b). The cell lines HL-60, U937 (monocytes),

Jurkat (T-cell leukaemia) and ECRF-24 (immortalized HUVEC) were all negative for properdin binding (Figure 3c).

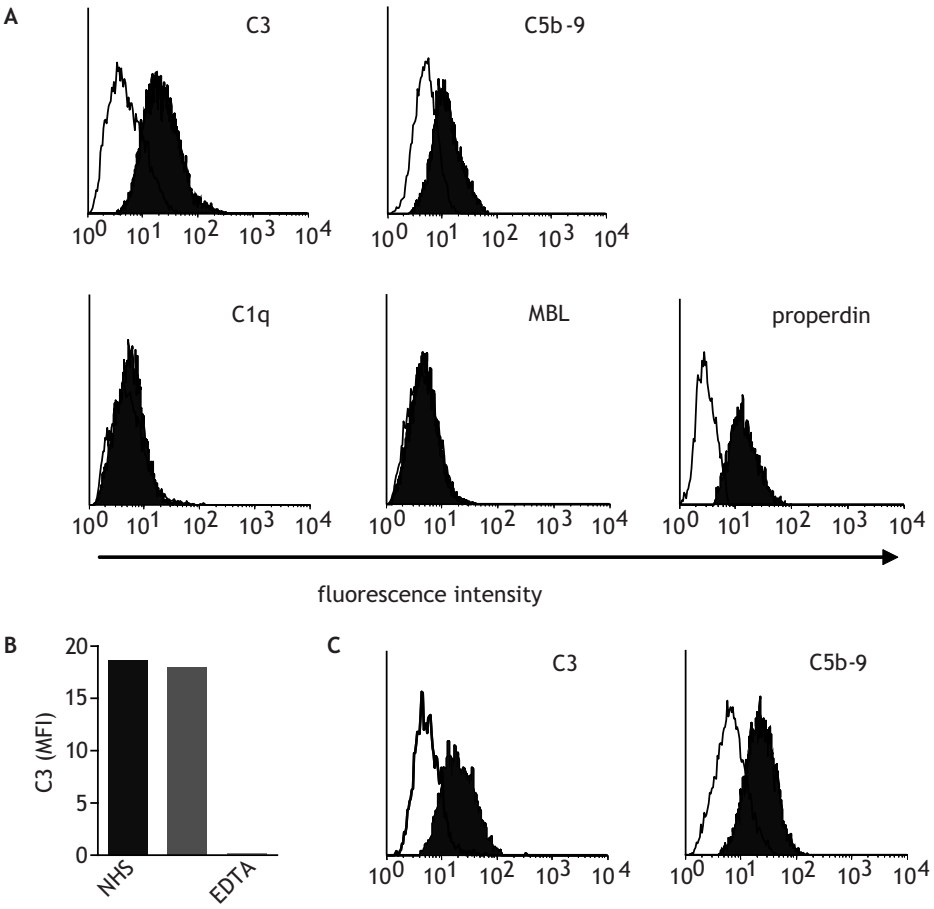

Figure 2. Complement activation by HK-2 cells. (**A**) HK-2 cells were incubated with 20% normal human serum (NHS). C3, C5b-9, C1q, mannan-binding lectin (MBL) and properdin binding (filled histograms) were assessed on the cells using the mAbs RFK22, AE11, 2204, 3E7 and a polyclonal rabbit anti-properdin antibody, respectively. Open histograms show staining on cells that were not exposed to serum. (**B**) C3 deposition was assessed on HK-2 cells after incubation with 20% human serum in the presence or absence of 5mM Mg EGTA or 10 mM EDTA. Results are expressed as the mean fluorescence intensity, MFI. (**C**) C3 and C5b-9 deposition on HK-2 cells after exposure to 20% C4-depleted human serum.

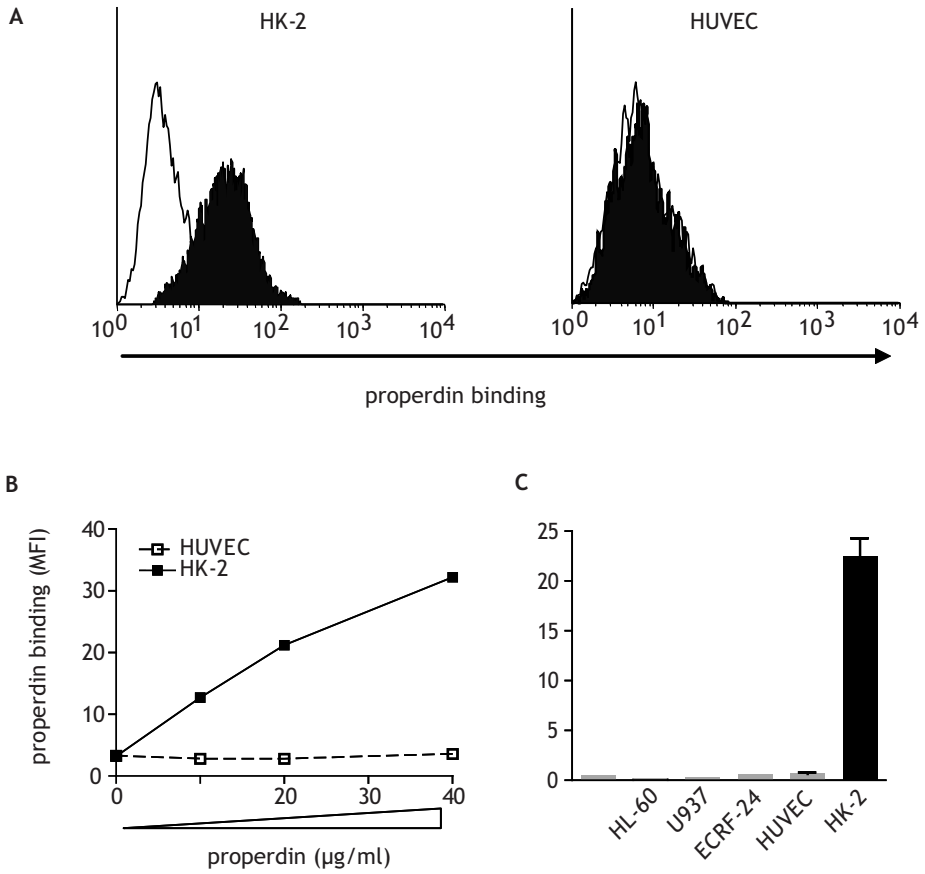

Figure 3. Properdin binding to HK-2 cells. (**A**) HK-2 cells and human umbilical vein endothelial cells (HUVEC) were incubated with 20 µg/ml purified human properdin. Binding of properdin (filled histograms) was detected with a polyclonal rabbit anti-human properdin antibody followed by goat anti-rabbit conjugated with PE. As a negative control, staining with both primary and secondary antibody was performed on cells that were not exposed to properdin (open histograms). (**B**) Dose-dependent binding of properdin to HK-2 and HUVEC is shown as the mean fluorescence intensity (MFI). Data are representative for two individual experiments. (**C**) Binding of properdin (shown as the mean fluorescence intensity, MFI) to the cell lines Jurkat, HL-60, U937, ECRF-24, HUVEC and HK-2. Data are expressed as the mean ± SD of three independent experiments.

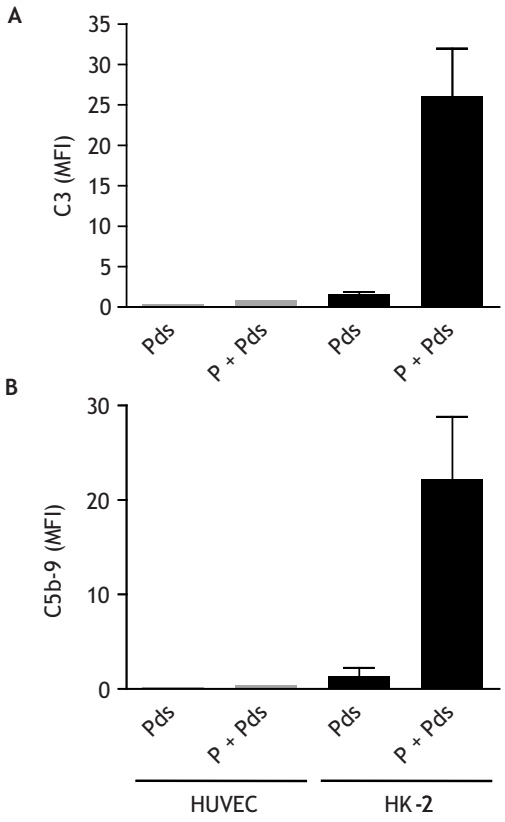

Figure 4. Properdin-dependent complement activation. HK-2 cells and human umbilical vein endothelial cells (HUVEC) were pre-incubated with properdin (P, 20 µg/ml), washed and subsequently exposed to 5% properdin-depleted human serum (Pds). (**a**) C3 and (**b**) C5b-9 deposition (shown as the mean fluorescence intensity, MFI) was detected on the cells using the mAbs RFK22 and AE11, respectively. The results are expressed as the mean ± SD of three independent experiments.

Properdin binding is a focal point for alternative pathway activation on HK-2 cells

Next, we investigated whether properdin, after binding to the tubular surface, acts as a focal point for local amplification of the alternative pathway of complement. To demonstrate properdin-dependent complement activation, deposition of C3 and C5b-9 was assessed on HK-2 cells and HUVEC incubated with properdin-deficient normal human serum, with and without pre-incubation of the cells with purified properdin. HK-2 cells incubated with properdin-depleted serum show a strongly reduced C3 deposition compared to cells exposed to normal human serum. This is accompanied by a strong reduction of C5b-9 deposition. Complement activation was restored completely on cells that had been pre-incubated with properdin, prior to exposure to properdin-deficient serum (Figure 4a and b). HUVEC showed no significant

complement activation, both with and without pre-incubation with purified properdin. This indicates that properdin, bound to the cellular surface of HK-2, initiates and targets the amplification of the complement cascade to the surface of tubular cells.

To confirm that complement activation on HK-2 is properdin-dependent, cells were pre-exposed to different concentrations of purified properdin, ranging from 2,5 to 40 µg/ml, before incubation with 5% normal human serum. Properdin was shown to increase the deposition of both C3 and C5b-9 on HK-2 in a dose-dependent way (Figure 5a). Similar dose-dependent effects were detected when increasing concentrations of properdin were added prior to incubation with C4-depleted human serum (Figure 5b).

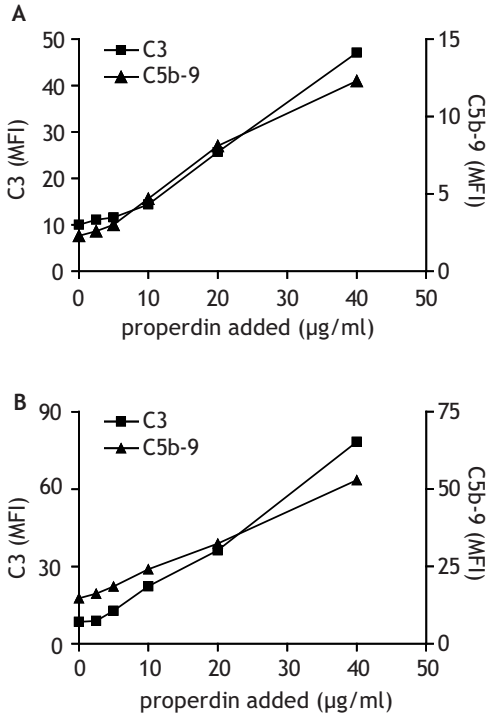

Figure 5. Dose-dependent effect of properdin on complement deposition. HK-2 cells were pre-incubated with increasing concentrations of human properdin. After extensive washing, cells were exposed to (**A**) 5% normal human serum or (**B**) 5% C4-depleted human serum. Complement deposition (expressed as the mean fluorescence intensity, MFI) was assessed by flow cytometry using mAbs RFK22 and AE11 for staining of C3 and C5b-9, respectively. Results represent one out of two experiments.

Properdin binding and complement activation on PTEC

In order to test whether the PTEC cell line HK-2 is representative for primary PTEC lines, properdin binding and properdin-dependent complement activation was assessed on primary PTEC cultures. As shown in Figure 6a, properdin binding on PTEC is comparable to HK-2 (Figure 3a). The six tested PTEC cell lines showed variability in properdin binding (Figure 6b). None of the three HUVEC cell lines showed significant binding of properdin. However, the extent of properdin binding to PTEC was strongly correlated with the level of C3 deposition on these cells, r = 0.96 / p = 0.002 (Figure 6c).

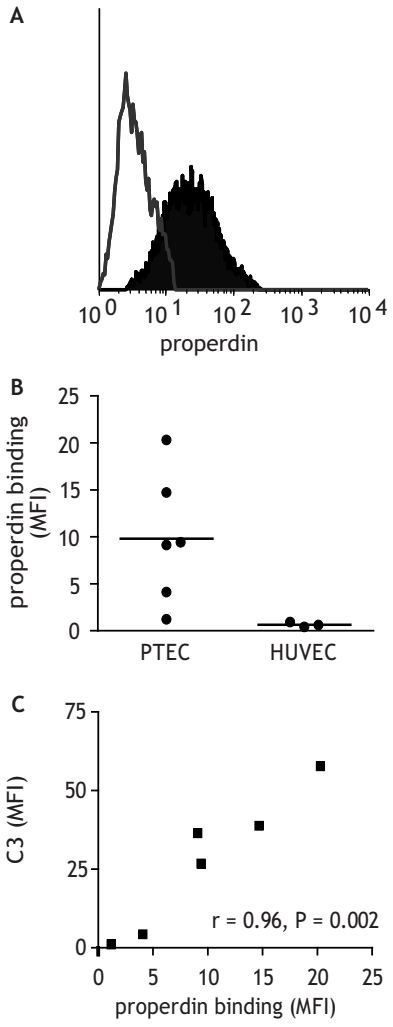

Figure 6. Properdin-mediated complement fixation on primary PTEC. (**A**) Primary proximal tubular epithelial cell (PTEC) lines were analysed for properdin binding (filled histogram) by flow cytometry after incubation with 20 µg/ml purified human properdin. The open histogram shows staining on cells that were not incubated with properdin. (**B**) Binding of properdin to different PTEC and human umbilical vein endothelial cell (HUVEC) lines. Properdin binding is expressed as the mean fluorescence intensity (MFI). The background fluorescence (primary and secondary antibody without properdin pre-incubation) is subtracted for each cell line individually. (**C**) Properdin binding and resulting properdin-dependent complement activation was tested by incubating the cells with 5% properdin-depleted human serum (Pds) after pre-exposure to 20 µg/ml purified properdin. Properdin binding and C3 deposition is shown as the mean fluorescence intensity (MFI). The association between properdin binding and the level of C3 deposition was analysed by calculating the Pearson correlation coefficient.

Discussion

In the present study, we show that properdin binds to the surface of viable PTEC. Properdin binding serves as a focal point for local amplification of the alternative pathway of complement on PTEC and explains the complement activating capacity of these cells.

It has been known for a long time that the apical surface of human proximal tubular epithelial cells activates the complement system *in vitro* and *in vivo* via the alternative pathway [13;14]. In patients suffering from chronic proteinuric renal disease, deposition of complement along the tubular brush border is accompanied by tubulointerstitial injury and progressive loss of renal function. Experimental models of non-selective proteinuria provide further evidence for the role of tubular complement activation in mediating tubulointerstitial injury [17;19]. C6 deficiency protects kidney function in the remnant kidney model as well as in the puromycin-induced model of nephrotic syndrome [16;18].

Although in physiological conditions complement components are not filtered through the glomerular barrier, several studies demonstrated the presence of complement activation products (CAP) in the urine of patients with nephrotic syndrome due to a variety of causes [10;37-39]. These studies showed a positive correlation between tubular C3 fixation and the excretion of complement components as well as complement activation products (including iC3b, Bb and C5b-9) in the urine. Interestingly, the level of urinary CAP excretion was significantly decreased after two weeks of oral sodiumbicarbonate administration [38;40]. The protective effect of bicarbonate was suggested to be due to lowering of the tubular ammonium concentration but may also be explained by a direct effect of increasing the urinary pH [41].

Despite extensive research, the mechanism of complement activation on the tubular brush border has not yet been fully elucidated. It was suggested that local ammonium reacts biochemically with the thioester of C3 and thereby acts as a C3 activator [20;21]. However, the addition of ammonium to serum only resulted in 15% increase in lysis of rabbit erythrocytes. This weak effect of ammonium on complement activation was only present in the lower concentration range. At higher concentrations, ammonium inhibited the alternative pathway. Recently, the activation of complement in proximal tubule cells was studied using proteinuric urine [41]. Increasing concentrations of ammonium resulted in an inhibition of complement activation. Ammonium excretion obviously does not fully explain the propensity of the renal tubule cells to activate the complement system.

Others have suggested that the lack of complement regulatory molecules on the apical surface of PTEC may explain the capacity of these cells to activate complement. Indeed, CD46 (membrane cofactor protein, MCP) only seems to be expressed on the basolateral surface of PTEC and CD55 (decay accelerating factor, DAF) could not be detected at all [42;43]. On the other hand CD59 is expressed abundantly on PTEC and surface expression of both CD46 and CD55 were detected on a PTEC cell line [44].

We suggest that the unique properdin binding capacity of PTEC critically controls the tubular complement activation in proteinuric states. In 1974, Sato *et al*. described that the damaging effect of intraluminally perfused normal rat serum on the rat kidney proximal tubule could be abolished by pre-incubating the serum with a brush border membrane fraction [45]. Possibly the effect of pre-inbcubation with the brush border membrane fraction is explained by its capacity to absorb properdin.

It was recently re-emphasized that properdin, the only known naturally occurring positive regulator of complement, can act as a focal point for alternative pathway amplification [27;28], thereby directing complement activation to the cell surface of apoptotic and necrotic cells [29;46]. Several decades before, Pillemer *et al*. suggested that properdin might also interact directly with target surfaces [23;31]. Likewise, we hypothesized that properdin might be the activator of the alternative pathway on the tubular brush border by interacting with molecules present on the cell membrane.

At the moment, the ligand on PTEC that mediates the interaction with properdin has not yet been identified. Properdin has been shown to bind to surface-bound C3b via one of its subunits followed by the assembly of the alternative pathway convertase at the ligand-binding sites of the adjoining subunits [27]. At the moment we can not fully exclude that properdin binds to PTEC via cell-bound C3b that is derived from endogenously produced and activated C3. Although C3b is undetectable by flow cytometry on PTEC, it might be present below the detection limit. On the other hand, it seems unlikely that significant amounts of C3b are present on quiescent cells. Recent data suggest that properdin also binds to the glycosphingolipid sulfatide [47]. The presence of sulfatide on the brush border of the tubules has been demonstrated in the rat kidney [48]. It is likely that these molecules are also expressed on the tubules in the human kidney, where they may mediate properdin binding to PTEC.

The mechanism by which a sublytic dose of C5b-9 on PTEC leads to tubular damage and subsequent tubulointerstitial fibrosis is thought to be via activation of proinflammatory and fibrogenic pathways [4]. Insertion of C5b-9 into the cell membrane of PTEC results in the production of proinflammatory cytokines and collagen synthesis. Interestingly, PTEC have been shown to synthesize a functional

alternative pathway of complement, which is capable of activating the cells [49]. This intratubular complement activation is tightly regulated and probably plays a role in protecting the kidney from urinary tract infections. Since the apical tubular surface does not come into contact with high concentrations of plasma proteins in normal physiology, protection against circulating complement is of less importance compared to circulating cells and the endothelium. However, in proteinuric renal disease, the tubules are exposed to filtered complement components. In these circumstances, the complement activating capacity of PTEC is harmful, especially since the apical surface has virtually no protection against complement attack [42].

Our data show that properdin binding to the brush border is the rate-limiting step in tubular complement activation. Targeting the interaction between properdin and the tubular brush border might be a therapeutic approach for controlling tubulointerstitial injury, thereby preventing progressive loss of kidney function in patients with chronic proteinuric renal disease.

Acknowledgements

We thank dr. Anja Roos for helpful discussions.

Reference List

1. Lysaght MJ. Maintenance dialysis population dynamics: current trends and long-term implications. *J Am Soc Nephrol* 2002; 13 Suppl 1:S37-S40.
2. Meguid EN, Bello AK. Chronic kidney disease: the global challenge. *Lancet* 2005; 365(9456):331-340.
3. Burton C, Harris KP. The role of proteinuria in the progression of chronic renal failure. *Am J Kidney Dis* 1996; 27(6):765-775.
4. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998; 339(20):1448-1456.
5. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993; 329(20):1456-1462.
6. Ruggenenti P, Perna A, Benini R, Bertani T, Zoccali C, Maggiore Q et al. In chronic nephropathies prolonged ACE inhibition can induce remission: dynamics of time-dependent changes in GFR. Investigators of the GISEN Group. Gruppo Italiano Studi Epidemiologici in Nefrologia. *J Am Soc Nephrol* 1999; 10(5):997-1006.
7. Wang Y, Chen J, Chen L, Tay YC, Rangan GK, Harris DC. Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein. *J Am Soc Nephrol* 1997; 8(10):1537-1545.
8. Tang S, Leung JC, Tsang AW, Lan HY, Chan TM, Lai KN. Transferrin up-regulates chemokine synthesis by human proximal tubular epithelial cells: implication on mechanism of tubuloglomerular communication in glomerulopathic proteinura. *Kidney Int* 2002; 61(5):1655-1665.
9. Wohlfarth V, Drumm K, Mildenberger S, Freudinger R, Gekle M. Protein uptake disturbs collagen homeostasis in proximal tubule-derived cells. *Kidney Int Suppl* 2003;(84):S103-S109.
10. Klajman A, Avital A, Myers BD. Renal handling of the third (C3) and fourth (C4) components of the complement system in the nephrotic syndrome. Nephron 1976; 16(5):333-343.
11. Tang S, Lai KN, Sacks SH. Role of complement in tubulointerstitial injury from proteinuria. *Kidney Blood Press Res* 2002; 25(2):120-126.
12. Abe K, Li K, Sacks SH, Sheerin NS. The membrane attack complex, C5b-9, up regulates collagen gene expression in renal tubular epithelial cells. *Clin Exp Immunol* 2004; 136(1):60-66.
13. Biancone L, David S, Della P, V, Montrucchio G, Cambi V, Camussi G. Alternative pathway activation of complement by cultured human proximal tubular epithelial cells. *Kidney Int* 1994; 45(2):451-460.
14. Camussi G, Stratta P, Mazzucco G, Gaido M, Tetta C, Castello R et al. In vivo localization of C3 on the brush border of proximal tubules of kidneys from nephrotic patients. *Clin Nephrol* 1985; 23(3):134-141.
15. Camussi G, Rotunno M, Segoloni G, Brentjens JR, Andres GA. In vitro alternative pathway activation of complement by the brush border of proximal tubules of normal rat kidney. *J Immunol* 1982; 128(4):1659-1663.
16. Nangaku M, Pippin J, Couser WG. C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys. *J Am Soc Nephrol* 2002; 13(4):928-936.

17. Morita Y, Nomura A, Yuzawa Y, Nishikawa K, Hotta N, Shimizu F et al. The role of complement in the pathogenesis of tubulointerstitial lesions in rat mesangial proliferative glomerulonephritis. *J Am Soc Nephrol* 1997; 8(9):1363-1372.
18. Nangaku M, Pippin J, Couser WG. Complement membrane attack complex (C5b-9) mediates interstitial disease in experimental nephrotic syndrome. *J Am Soc Nephrol* 1999; 10(11):2323-2331.
19. He C, Imai M, Song H, Quigg RJ, Tomlinson S. Complement inhibitors targeted to the proximal tubule prevent injury in experimental nephrotic syndrome and demonstrate a key role for C5b-9. *J Immunol* 2005; 174(9):5750-5757.
20. Nath KA, Hostetter MK, Hostetter TH. Pathophysiology of chronic tubulo-interstitial disease in rats. Interactions of dietary acid load, ammonia, and complement component C3. *J Clin Invest* 1985; 76(2):667-675.
21. Nath KA, Hostetter MK, Hostetter TH. Ammonia-complement interaction in the pathogenesis of progressive renal injury. *Kidney Int Suppl* 1989; 27:S52-S54.
22. Walport MJ. Complement. First of two parts. *N Engl J Med* 2001; 344(14):1058-1066.
23. Pillemer L, Blum L, Lepow IH, Ross OA, Todd EW, Wardlaw AC. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science* 1954; 120(3112):279-285.
24. Schwaeble WJ, Reid KB. Does properdin crosslink the cellular and the humoral immune response? *Immunol Today* 1999; 20(1):17-21.
25. Smith CA, Pangburn MK, Vogel CW, Muller-Eberhard HJ. Molecular architecture of human properdin, a positive regulator of the alternative pathway of complement. *J Biol Chem* 1984; 259(7):4582-4588.
26. Fearon DT, Austen KF. Properdin: binding to C3b and stabilization of the C3b-dependent C3 convertase. *J Exp Med* 1975; 142(4):856-863.
27. Hourcade DE. The role of properdin in the assembly of the alternative pathway C3 convertases of complement. *J Biol Chem* 2006; 281(4):2128-2132.
28. Spitzer D, Mitchell LM, Atkinson JP, Hourcade DE. Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. *J Immunol* 2007; 179(4):2600-2608.
29. Kemper C, Mitchell LM, Hourcade DE. Properdin: Cell death's little helper? *Mol Immunol* 2007; 107(Abstract):Abstract.
30. Kimura Y, Miwa T, Zhou L, Song WC. Activator-specific requirement of properdin in the initiation and amplification of the alternative pathway complement. *Blood* 2007.
31. WARDLAW AC, PILLEMER L. Inhibition of the bactericidal activity of the properdin system by zymosan and other properdin binding agents. *J Immunol* 1959; 82(4):313-318.
32. Ryan MJ, Johnson G, Kirk J, Fuerstenberg SM, Zager RA, Torok-Storb B. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. *Kidney Int* 1994; 45(1):48-57.
33. van Kooten C, Gerritsma JS, Paape ME, van Es LA, Banchereau J, Daha MR. Possible role for CD40-CD40L in the regulation of interstitial infiltration in the kidney. *Kidney Int* 1997; 51(3):711-721.

34. Taekema-Roelvink ME, van Kooten C, Heemskerk E, Schroeijers W, Daha MR. Proteinase 3 interacts with a 111-kD membrane molecule of human umbilical vein endothelial cells. *J Am Soc Nephrol* 2000; 11(4):640-648.
35. Fontijn R, Hop C, Brinkman HJ, Slater R, Westerveld A, van Mourik JA et al. Maintenance of vascular endothelial cell-specific properties after immortalization with an amphotrophic replication-deficient retrovirus containing human papilloma virus 16 E6/E7 DNA. *Exp Cell Res* 1995; 216(1):199-207.
36. Nauta AJ, Trouw LA, Daha MR, Tijsma O, Nieuwland R, Schwaeble WJ et al. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *Eur J Immunol* 2002; 32(6):1726-1736.
37. Lange K, Wenk EJ. Complement components in the sera and urines of patients with severe proteinurias. *Am J Med Sci* 1954; 228(4):448-453.
38. Morita Y, Ikeguchi H, Nakamura J, Hotta N, Yuzawa Y, Matsuo S. Complement activation products in the urine from proteinuric patients. *J Am Soc Nephrol* 2000; 11(4):700-707.
39. Ogrodowski JL, Hebert LA, Sedmak D, Cosio FG, Tamerius J, Kolb W. Measurement of SC5b-9 in urine in patients with the nephrotic syndrome. *Kidney Int* 1991; 40(6):1141-1147.
40. Rustom R, Grime JS, Costigan M, Maltby P, Hughes A, Taylor W et al. Oral sodium bicarbonate reduces proximal renal tubular peptide catabolism, ammoniogenesis, and tubular damage in renal patients. *Ren Fail* 1998; 20(2):371-382.
41. Peake PW, Pussell BA, Mackinnon B, Charlesworth JA. The effect of pH and nucleophiles on complement activation by human proximal tubular epithelial cells. *Nephrol Dial Transplant* 2002; 17(5):745-752.
42. Ichida S, Yuzawa Y, Okada H, Yoshioka K, Matsuo S. Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int* 1994; 46(1):89-96.
43. Blok VT, Daha MR, Tijsma OM, Weissglas MG, van den Broek LJ, Gorter A. A possible role of CD46 for the protection in vivo of human renal tumor cells from complement-mediated damage. *Lab Invest* 2000; 80(3):335-344.
44. Li K, Feito MJ, Sacks SH, Sheerin NS. CD46 (membrane cofactor protein) acts as a human epithelial cell receptor for internalization of opsonized uropathogenic Escherichia coli. *J Immunol* 2006; 177(4):2543-2551.
45. Sato K, Ullrich KJ. Serum-induced inhibition of isotonic fluid absorption by the kidney proximal tubule. II. Evidence that complement is involved. *Biochim Biophys Acta* 1974; 354(2):182-187.
46. Xu W, Berger SP, Trouw LA, de Boer HC, Schlagwein N, Mutsaers C et al. Properdin binds to late apoptotic and necrotic cells independently of c3b and regulates alternative pathway complement activation. *J Immunol* 2008; 180(11):7613-7621.
47. Holt GD, Pangburn MK, Ginsburg V. Properdin binds to sulfatide [Gal(3-SO4)beta 1-1 Cer] and has a sequence homology with other proteins that bind sulfated glycoconjugates. *J Biol Chem* 1990; 265(5):2852-2855.
48. Trick D, Decker J, Groene HJ, Schulze M, Wiegandt H. Regional expression of sulfatides in rat kidney: immunohistochemical staining by use of monospecific polyclonal antibodies. *Histochem Cell Biol* 1999; 111(2):143-151.
49. Peake PW, O'Grady S, Pussell BA, Charlesworth JA. C3a is made by proximal tubular HK-2 cells and activates them via the C3a receptor. *Kidney Int* 1999; 56(5):1729-1736.