Endogenous tissue-type plasminogen activator is protective during ascending urinary tract infection

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Abstract

Background. Acute pyelonephritis is one of the most common bacterial infections. Tissue-type plasminogen activator (tPA) is a potent fibrinolytic agent, but can play a role in inflammatory processes as well.

Methods. We induced pyelonephritis in tPA−/− and C57BL/6 wild-type (WT) mice by intravesical inoculation with 1010 CFU uropathogenic Escherichia coli 1677. The mice were killed after 24 and 48 h, after which bacterial outgrowth and cytokine levels in kidney homogenates were determined. Influx of neutrophils was quantified by myeloperoxidase-ELISA. Neutrophil phagocytosis and oxidative burst were measured.

Results. The tPA−/− kidneys contained significantly higher numbers of E. coli CFU, accompanied by higher levels of interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α). The number of infiltrating neutrophils was similar in tPA−/− and WT mice at both time points, suggesting that tPA−/− neutrophils have a lower ability to eliminate E. coli. Phagocytosis of E. coli organisms was not diminished in tPA−/− neutrophils. Interestingly, tPA−/− neutrophils showed a significantly lower ability to generate an oxidative burst reaction upon stimulation with E. coli than WT neutrophils. Incubation with recombinant tPA reversed this effect completely.

Conclusions. These results show that deletion of the tPA-gene in mice leads to lower bactericidal potential of tPA−/− neutrophils, which results in significantly more bacterial outgrowth during experimental pyelonephritis.

Keywords: fibrinolytic system; neutrophil; pyelonephritis; oxidative burst; tissue-type plasminogen activator

Introduction

Urinary tract infections (UTI), most frequently caused by Gram-negative Escherichia coli, are among the most common bacterial infections, affecting ∼50% of women at one point in their lifetime [1], while recurrent UTI afflict ∼10% of women [1]. Also, UTI form a considerable clinical problem for paediatricians since 8% of girls and 2% of boys under the age of 7 develop acute pyelonephritis [2]. When left untreated, pyelonephritis may lead to renal fibrosis and subsequent end-stage renal failure [3].

The dynamical balance between bacterial virulence factors and host defence mechanisms determines the outcome of UTI. Virulence factors that are linked to uropathogenicity of E. coli comprise type 1 fimbriae, P fimbriae, haemolysin and aerobactin. Fimbriae are adhesins that assist the attachment of E. coli to the urothelium (type 1 fimbriae) and to glycolipids on resident kidney cells (P fimbriae). Haemolysin assists E. coli tissue invasion and lysis of host leukocytes, whereas aerobactin is a siderophore that ensures bacterial metabolism in low-iron environments such as urine and serum [4].

Host defence mechanisms against UTI include non-specific factors such as regular bladder voiding and antimicrobial peptides such as β-defensin 1, which is expressed by tubular epithelial cells and has been reported to be protective against UTI [5]. Upon adhesion of uropathogenic bacteria to the urothelium, complement activation and generation of pro-inflammatory cytokines and chemokines lead to recruitment of neutrophils that ingest and kill E. coli organisms [6]. Killing of bacteria by neutrophils depends on the capacity of these cells to produce an oxidative burst. The generation of reactive oxygen species (ROS) results from activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a multiprotein enzyme complex that transfers a single electron from NADPH to oxygen, resulting in the production of superoxide (O2−) and subsequent generation of hydrogen peroxide (H2O2) [7].

Neutrophil myeloperoxidase (MPO) reacts with O2− and
H$_2$O$_2$ to generate highly reactive hypochlorous acid (HOCI) [8].

The fibrinolytic system consists of two serine proteases, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) that both facilitate the conversion of plasminogen into plasmin, which is the key protease in the fibrinolytic cascade [9]. In the kidney, tPA is constitutively expressed by endothelial cells, glomerular cells and epithelial cells of the distal collecting duct [10]. Under inflammatory conditions, tPA can be expressed by proximal tubules as well [11].

In recent years, it has become increasingly clear that components of the fibrinolytic system can exert functions that exceed their familiar proteolytic tasks. Recently, it has been demonstrated that tPA has the ability to initiate cell signalling cascades in renal fibroblasts after interaction with the low-density lipoprotein receptor-related protein-1 (LRP-1) [12]. The tPA-LRP-1 interaction resulted in tyrosine phosphorylation on the beta subunit of LRP-1, which was followed by the activation of Mek1, Erk-1/2 and subsequent induction of matrix metalloproteinase 9 (MMP-9) gene expression [12]. In this way tPA has proven to act in a cytokine-like manner, in a plasmin-independent fashion. In a previous study on renal ischaemia-reperfusion injury, we have shown that tPA deficiency resulted in reduced neutrophil influx, leading to quicker recovery of renal function after ischaemia [11]. Again, this effect was independent from proteolytic activity. Furthermore, we were able to define endogenous tPA as an important protective factor during E. coli-induced abdominal sepsis [13].

In order to investigate the in vivo role of tPA during UTI, we induced acute pyelonephritis in tPA-deficient mice by intravesical inoculation with uropathogenic E. coli. In the present study, we demonstrate that deletion of the tPA gene in mice leads to higher bacterial load during experimental pyelonephritis, partly caused by an impaired ability to generate an oxidative burst by tPA$^{-/-}$ neutrophils.

**Methods**

**Mice and experimental protocol**

The tPA$^{-/-}$ mice on a C57BL/6J background were purchased from The Jackson Laboratory (Bar Harbor, Maine) and bred in the institutional animal facilities. Age- and weight-matched C57Bl/6 mice (Charles River, Maastricht, The Netherlands) served as a wild-type (WT) control.

UTI was induced as described previously [14,15]. *E. coli* 1677, isolated from a uroseptic patient, was donated by Dr W. J. Hopkins (University of Wisconsin Medical School, Madison, WI, USA). This strain has virulence factors that include type 1 and Pfimbriae, haemolysin and aerobactin [16]. This specific serotype has proven uropathogenicity in mice [14,16]. Bacteria were cultured for 16 h at 37°C in the trypticase soy broth (TSB). After dilution 1:100 in fresh TSB, the suspension was grown for 3 h to midlogarithmic phase. *E. coli* were washed three times in sterile PBS. The bacteria were resuspended in PBS at a concentration of $1 \times 10^{11}$ colony-forming units (CFU)/mL, as determined by plating 10-fold serial dilutions of the suspension on blood agar plates. Acute pyelonephritis was induced under general anesthesia [0.07 mL/10 g mouse of FFM mixture, containing 1.25 mg/mL midazolam (Roche, Mijdrecht, The Netherlands), 0.08 mg/mL fentanyl citrate and 2.5 mg/mL flumisonone (Janssen Pharmaceutica, Beerse, Belgium)] in 8- to 10-week-old female mice. Hundred microlitres of bacterial suspension ($1 \times 10^{10}$ CFU) was administered transurethrally through a 0.55 mm catheter (Abbott, Zwolle, The Netherlands). Sham control mice underwent the same procedure with administration of 100 µL of sterile PBS instead of bacterial suspension. Mice were killed 24 h (n = 7 per group) and 48 h (n = 8 per group) after the procedure by exsanguination under general anaesthesia. The choice of these time points was based on the literature and on our own broad experience with this model [14–17]. Under normal circumstances, C57Bl/6 mice have the ability to clear an infection with this *E. coli* strain during 48 h [16]. White blood cell counts (WBC) in peripheral blood were determined using a haemocytometer (Beckman Coulter, Fullerton, CA, USA). The Committee on Use and Care of Animals of the Academic Medical Center at the University of Amsterdam approved all experiments. Animal experimentation guidelines were followed in all experiments.

**Determination of bacterial outgrowth**

Bacterial load was determined in renal tissue and blood as described earlier [14,15]. One-half of the left kidney from each mouse was homogenized in four volumes of sterile saline with a tissue homogenizer that was cleaned with 70% ethanol after each homogenization. Serial 10-fold dilutions were made in sterile saline, and 50 µL volumes of kidney homogenate and blood were plated onto blood agar plates, which were incubated at 37°C for 16 h, after which *E. coli* CFU were counted.

**Histology and determination of fibrin deposition**

The remaining part of the left kidney was fixed in 10% formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin in a standard fashion. For fibrin immunostaining 4-µm sections were pretreated with 0.1% trypsin (Sigma) at 37°C for 30 min. Endogenous peroxidase activity was quenched by 0.1% Na$_2$O$_2$/0.03% H$_2$O$_2$ and free protein-binding sites were blocked with 10% normal goat serum. After incubation with biotin-labelled antimouse fibrinogen (IXELL; Accurate Chemical & Scientific, Westbury, NY, USA) slides were incubated with a streptavidine/biotinylated horseradish peroxidase (HRP) mixture (StreptABComplex, DAKO, Glostrup, Denmark). The bound HRP was visualized using 3,3-diamino-benzidine tetrachloride (DAB, Sigma). Fibrin deposits in renal tissue were quantified by western blot analysis as described previously [18,19]. After electrophoresis of kidney homogenates by SDS–PAGE, proteins were blotted onto a PVDF membrane (Immobilon-P, Millipore, Bredford, MA, USA). The 56-kDa fibrin β-chains were detected with a monoclonal anti-human fibrin antibody (59D8, Boston Research Services, Winchester, MA, USA) that specifically recognizes fibrin β-chains, and not fibrinogen [20].
Cytokine and MPO measurements

The right kidney of each mouse was homogenated in PBS containing 1% Triton X-100, 1 mM EDTA (Merck) and 1% protease inhibitor cocktail (P8340, Sigma). Levels of interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), keratinocyte-derived chemokine (KC), macrophage inflammatory protein-2 (MIP-2) and mouse MPO in kidney homogenates were measured by specific ELISA according to the instructions of the manufacturer (IL-1β and TNF-α: R&D systems, Minneapolis, MN; MPO: HyCult biotechnologies, Uden, The Netherlands). Total protein content of the samples was determined according to the method of Bradford [21], using a commercially available protein assay kit (Bio-Rad, Hercules, CA, USA).

Real-time quantitative PCR analysis

Real-time quantitative (Q) PCR analysis was performed using RNA obtained from kidneys of WT and tPA−/− mice, at T = 24 h. Total RNA was isolated using TRIzol® LS Reagent (Invitrogen—Life Technologies, The Netherlands), following the manufacturer’s instructions. The concentration and purity of RNA were determined spectrophotometrically at 260/280 nm. Five micrograms of total RNA was reverse-transcribed into cDNA using oligo dT primers (Roche). Primer sets used were for tPA: forward ATGAGGCATCGTCTCCATTC, reverse CATGTTGTTC GTTCTTTGAACGTCTCTGTCC; IL-1β: forward CTGCAGCTGGAGAGTGTTGGAT, reverse GCTTGTGCTCTGCTTGTGAG; TNF-α: forward TCTAGAGAAACCAACCAAGTG, reverse CCTCTGTCCTTGAAGAGACC; KC: forward ATATGGGGCTTCTTACCTTTTAACC, reverse AG TCTTTTGAAACGTCTCTGTCC; MIP-2: forward CCTGGTTCAGAAAAATCATCC, reverse CTTC GTTGGAGGGACGC. TBP was used as housekeeping gene: forward GGAGAATCATGGACCAGAAC, reverse GATGGGAATTCCAGGAGTCA. QPCR was performed with a LightCycler® LC480 (Roche). For the PCR, a master mixture was prepared on ice, containing per sample 1 µL of cDNA, 1 µL of FastStart Reaction Mix SYBR Green I (Roche Applied Science, Indianapolis, IN, USA), 0.5 µL of 10 µM primers and 1.6 µL of 25 mM MgCl2. After the reaction mixture was loaded into a glass capillary tube, the cycling conditions were carried out as follows: initial denaturation at 95°C for 6 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 55–60°C for 5–10 s and extension at 72°C for 8–12 s. The temperature transition rate was set at 20°C/s. The fluorescent product was measured by a single acquisition mode at 72°C after each cycle. Quantification of data was performed using standard Light Cycler analysis software. The amount of amplified tPA and cytokine products was divided by the amount of TBP for each sample.

Phagocytosis assay and oxidative burst

Expression of eGFP was induced in E. coli strains Top-10 and DH10B (both Invitrogen, Breda, The Netherlands), by transfection with pBRR1MCS-5-eGFP (clone pMP2463) [22], which was kindly provided by Professor H. Spaink (Leiden University, Leiden, The Netherlands). Bacteria were grown under high selective pressure (40 µg/mL gentamycin, Sigma). GFP expression was confirmed by flow cytometry and fluorescent microscopy. The phagocytosis capacity of WT and tPA−/− neutrophils was determined by flow cytometry. In short, GFP-E. coli (equivalent to 50 × 106 CFU) was added to 50 µL of heparinized full blood from WT or tPA−/− mice and allowed to incubate for 5, 10, 15 or 20 min, vigorously shaking in a water bath at 37°C. Phagocytosis was stopped by immediately transferring the samples to ice. Erythrocytes were lysed using a hypotonic lysis buffer and neutrophils were labelled using anti-Ly6-G-APC (Pharmingen). To exclude the influence of cell surface-adhering bacteria extensive washing steps were included. Samples were washed with an ice-cold FACS buffer after which the degree of phagocytosis was determined using a flow cytometer (FACScalibur®, Becton Dickinson, Franklin Lakes, NJ, USA). Results are expressed as mean fluorescence intensity (MFI) per neutrophil.

Oxidative burst of neutrophils was assessed by dihydrorhodamine 123 (Sigma) measurement according to Kampen et al. [23]. In short, circulating cells (50 µL whole blood form WT or tPA−/− mice) were loaded with 1.5 mg/mL dihydrorhodamine 123 for 30 min. Neutrophils were stimulated by addition of 50 × 106 CFU E. coli 1677/mL for 30 min, with or without recombinant murine tPA (Molecular Innovations Inc., Southfield, MI, USA) at a concentration of 100 ng/mL. After red blood cell lysis, the conversion of dihydrochloride was determined by flow cytometry.

Statistical analysis

All data are presented as means ± standard error of the mean (SEM). Data were analysed by the Mann–Whitney U-test or unpaired Student’s t-test when appropriate. P < 0.05 was considered to represent a statistically significant difference.

Results

tPA is upregulated during urinary tract infection

To gain insight in the production of tPA during E. coli-induced acute pyelonephritis, we measured renal tPA concentration at mRNA level during acute pyelonephritis. At T = 24 h after infection tPA mRNA levels were significantly elevated, compared to sham mice (Figure 1).

tPA−/− mice display higher bacterial load than WT mice

To determine the role of tPA in UTI, acute pyelonephritis was induced in tPA−/− and WT mice, and renal bacterial loads were determined 24 and 48 h after infection. At both time points, tPA−/− mice showed a significantly higher number of E. coli CFU in kidney homogenates than WT mice (Figure 2), indicating that tPA−/− mice had a seriously impaired ability to clear E. coli-caused pyelonephritis. Blood cultures remained negative in all mice.
**tPA**<sup>−/−</sup> mice have higher levels of **IL-1β** and **TNF-α** during pyelonephritis

In order to investigate whether increased bacterial outgrowth in kidneys of **tPA**<sup>−/−</sup> mice was accompanied by higher levels of pro-inflammatory cytokines and chemokines, we determined levels of **IL-1β**, **TNF-α**, **KC** and **MIP-2** in kidney homogenates by QPCR and ELISA.

Both WT and **tPA**<sup>−/−</sup> kidneys showed upregulation of **IL-1β**, **TNF-α** and **MIP-2** mRNA at 24 h. As shown in Table 1, **tPA**<sup>−/−</sup> kidneys contained higher amounts of **IL-1β** and **TNF-α** mRNA than WT kidneys.

**tPA**<sup>−/−</sup> neutrophils have a lower bactericidal potential than WT neutrophils

Although **tPA**<sup>−/−</sup> and WT mice showed the same numbers of intra-renal neutrophils, **tPA**<sup>−/−</sup> mice displayed higher numbers of **E. coli** CFU, suggesting that **tPA**<sup>−/−</sup> neutrophils had an impaired ability to eliminate **E. coli**. In order to further investigate this, a phagocytosis assay was performed with **tPA**<sup>−/−</sup> and WT neutrophils. As shown in Figure 4, **tPA**<sup>−/−</sup> and WT neutrophils had the same ability to phagocytose two different **E. coli** strains. Both types of neutrophils

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**Table 1.** Gene expression of pro-inflammatory cytokines by QPCR

<table>
<thead>
<tr>
<th>Gene expression/TBP</th>
<th><strong>tPA</strong>&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>WT</th>
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<tr>
<td><strong>IL-1β</strong></td>
<td>4.4 ± 1.3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.79 ± 0.58</td>
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<tr>
<td><strong>TNF-α</strong></td>
<td>14.95 ± 1.14&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.97 ± 0.10</td>
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<tr>
<td><strong>MIP-2</strong></td>
<td>3.9 ± 5.2</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td><strong>KC</strong></td>
<td>0.09 ± 0.07</td>
<td>0.10 ± 0.07</td>
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Data are expressed as amount of mRNA relative to housekeeping gene **TBP**; mean ± SEM; <sup>+</sup><i>P</i> < 0.05.

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**Table 2.** Higher cytokine levels in **tPA**<sup>−/−</sup> kidneys

<table>
<thead>
<tr>
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<th><strong>tPA</strong>&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>WT</th>
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<tbody>
<tr>
<td><strong>IL-1β</strong></td>
<td>5.7 ± 1.3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td><strong>TNF-α</strong></td>
<td>11.6 ± 1.7&lt;sup&gt;+&lt;/sup&gt;</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td><strong>KC</strong></td>
<td>17.9 ± 1.3</td>
<td>15.7 ± 1.6</td>
</tr>
<tr>
<td><strong>MIP-2</strong></td>
<td>20.0 ± 1.2</td>
<td>22.1 ± 3.0</td>
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On **T** = 24 h and **T** = 48 h **tPA**<sup>−/−</sup> kidneys displayed significantly higher levels of **IL-1β** and **TNF-α** than WT kidneys (Table 2). In contrast, levels of **KC** and **MIP-2** did not differ significantly between **tPA**<sup>−/−</sup> and WT kidneys (Table 2).
Histology and renal myeloperoxidase levels. Photomicrographs of tPA−/− kidney (A) and WT kidney (B). Both tPA−/− and WT kidneys show a dense inflammatory infiltrate in the pyelum region. The infiltrate consists chiefly of neutrophils. T = 24 h after inoculation, H&E staining, original magnifications ×200. (C) At T = 24 h and T = 48 h after inoculation MPO levels were not different between tPA−/− (●) and WT (□) mice. Data expressed as mean ± SEM; Mann–Whitney U-test, P > 0.05.

WT and tPA−/− cells. Upon activation with uropathogenic E. coli, tPA−/− neutrophils displayed a diminished ability to produce an oxidative burst when compared to WT neutrophils (Figure 5). As shown in Figure 5, the addition of recombinant murine tPA reversed this effect completely.

Discussion

Acute pyelonephritis is one of the most commonly encountered infectious diseases and knowledge on its pathophysiology is still increasing. Numerous host factors have been implicated in host defence against E. coli pyelonephritis. Among the various components of the fibrinolytic system, only the uPA receptor (uPAR) has been implicated in host defence against UTI. Expression of uPAR is markedly elevated in urine and plasma of patients with culture-proven urosepsis [24]. In addition, we have shown that uPAR−/− mice experience more severe disease with higher bacterial outgrowth than WT mice during experimental pyelonephritis [15]. The present study shows that endogenous tPA is also involved in host defence against acute pyelonephritis. Indeed, tPA−/− mice have a seriously impaired bacterial clearance.

It has become increasingly clear that tPA can be an active modulator of inflammatory responses in a variety of
inflammatory conditions [11–13,25–30]. Only a very limited number of studies, however, deal with the involvement of tPA in host defence against micro-organisms. One report concerning a murine model of systemic *Mycobacterium avium* infection described no differences in total bacterial outgrowth between tPA−/− and WT mice although in tPA−/− mice dissemination of mycobacteria occurred earlier than in WT mice [31]. Recently, we described that endogenous tPA is an important protective factor during *E. coli*-induced abdominal sepsis [13]. After intraperitoneal inoculation, tPA−/− mice demonstrated higher bacterial load at the site of infection, enhanced *E. coli* dissemination and reduced survival. The results of the present study are in line with these findings.

The increased outgrowth of *E. coli* in tPA−/− kidneys was associated with higher levels of intra-renal IL-1β and TNF-α. It is likely that resident kidney cells contribute to cytokine production in this model. Indeed, after inoculation with uropathogenic *E. coli*, bladder and tubular epithelial cells have been shown to produce several pro-inflammatory cytokines and chemokines, among which IL-1β and TNF-α [32]. In a previous study we demonstrated that renal cytokine levels are directly correlated to the amount of NF-κB in this model [15]. Despite higher levels of pro-inflammatory cytokines in tPA−/− kidneys and higher numbers of circulating leukocytes in tPA−/− mice upon UTI, the amount of infiltrating neutrophils in tPA−/− and WT kidneys did not differ in this model. This finding is most probably related to the relatively impaired migration capacity of tPA−/− neutrophils and the similar levels of KC and MIP-2. The mouse CX3C chemokines KC and MIP-2 play a major role in the attraction of neutrophils during inflammation [33]. A relatively impaired migratory ability of tPA−/− neutrophils has also been observed in a model of renal ischaemia-reperfusion injury, in which tPA deficiency resulted in a diminished neutrophil infiltration into post-ischaemic renal tissue despite equal levels of pro-inflammatory cytokines and chemokines [11]. In a model of *E. coli*-induced peritonitis numbers of intra-peritoneal neutrophils were similar in tPA−/− and WT mice despite higher numbers of bacteria and higher cytokine levels in tPA−/− mice [13]. During thioglycolate-induced peritonitis, tPA−/− mice also demonstrated a reduced influx of neutrophils into the abdominal cavity. In addition, mice that over-expressed tPA showed significantly increased numbers of intra-peritoneal neutrophils during *E. coli* peritonitis relative to mice with normal tPA levels [13]. Taken together, these studies suggest that tPA actively favours neutrophil migration. In contrast with the abovementioned studies, in a model of sterile arthritis tPA−/− mice experienced more severe joint disease associated with increased fibrin deposition and higher numbers of infiltrating neutrophils [29,30]. Furthermore, in a model of crescentic glomerulonephritis, tPA−/− mice displayed more fibrin deposits, worse renal function and significantly higher numbers of intra-glomerular inflammatory cells [23]. Since fibrin has been implicated in the pathogenesis of both arthritis and glomerulonephritis [34,35], fibrin deposition parallels disease severity in these models. In the present study no appreciable fibrin deposits were present, suggesting that fibrin is not crucially involved in the pathophysiology of pyelonephritis. Furthermore, the abundant expression of uPA in the tubulointerstitial compartment of the kidney is likely to compensate fully for tPA deficiency [11].

Crucial steps in the clearance of bacterial infections are phagocytosis and subsequent killing of bacteria by means of an oxidative burst [6]. Here we show that tPA−/− and WT neutrophils have a similar capacity to phagocytose *E. coli*. Expression of α-selectin and β2-integrin—important determinants of leukocyte function—is also equal in WT and tPA−/− neutrophils (data not shown). However, upon stimulation with uropathogenic *E. coli* tPA−/− neutrophils display a significantly lower production of ROS than WT neutrophils, most probably resulting in higher numbers of surviving bacteria in our UTI model. Interestingly, the addition of recombinant tPA results in a complete restoration of the oxidative capacity of tPA−/− neutrophils. Previous reports on the effect of tPA on ROS production are inconsistent. *In vitro* administration of exogenous tPA can both enhance and reduce neutrophil ROS production upon induction by phorbol-12-myristate-13-acetate (PMA) [36,37]. Since PMA directly activates protein kinase C (PKC), thus bypassing cell surface receptors [38], these studies suggest that exogenous tPA can affect the non-receptor-mediated superoxide production cascade *in vitro* although its net effect remains unclear. In the present study, we use uropathogenic *E. coli* as a stimulus, which triggers the receptor-mediated pathway of superoxide generation.

Deficiency for uPA has been shown to result in a lower phagocytic potential of neutrophils, combined with a lower oxidative burst [39]. Upon neutrophil activation, uPA is released from intracellular vesicles into the extracellular compartment [40,41]. The uPA most probably regulates phagocytosis and ROS production through interaction with its highly specific cellular receptor uPAR, which by itself is crucially involved in neutrophil phagocytosis as well [15,39].

Activated neutrophils not only produce uPA but tPA as well, be it in smaller quantities [42–44]. Opposed to uPA, no specific tPA receptor has been described thus far. It is known that tPA can bind to the low-density LRP-1 and to annexin A2 although binding to these receptors occurs in a non-specific fashion. LRP-1 is a scavenging receptor that is crucially involved in the clearance of its numerous structurally different ligands but has the ability to act as a signalling receptor as well [45]. However, LRP-1 is not expressed by neutrophils [46], which makes it unlikely that LRP-1 is involved in the observed oxidative burst modulation.

The other tPA receptor, annexin A2, is a widely expressed cell membrane Ca²⁺/phospholipid-binding protein that is present on endothelial cells and on neutrophils as well [47–49]. Annexin A2 has the ability to function as a signalling receptor and has been implicated in neutrophil chemotaxis [49]. Recently, it was shown that interaction of tPA with Annexin A2 mediates activation of microglia, the immune cells of the brain, in a non-proteolytic fashion [50]. Although further research will be needed, interaction of tPA and annexin A2 on neutrophils might influence chemotaxis and possibly superoxide production.

The present study describes for the first time that endogenous tPA is critically involved in host defence
during *E. coli*-induced pyelonephritis, most likely by affecting neutrophil migration and neutrophil oxidative burst generation. Our data extend current knowledge on host-pathogen interactions during UTI and provide new insight into the manifold effects of tPA.

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Conflict of interest statement. None declared.

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