Kidney Dendritic Cells Induce Innate Immunity against Bacterial Pyelonephritis

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ABSTRACT

Dendritic cells (DCs) are the most abundant immune cells in the kidney and form an intricate network in the tubulointerstitium, suggesting that they may play an important role in interstitial infections such as pyelonephritis. Here, we optimized a murine pyelonephritis model by instilling uropathogenic Escherichia coli two times at a 3-hour interval, which produced an infection rate of 84%. By 3 hours after the second instillation, resident kidney DCs began secreting the chemokine CXCL2, which recruits neutrophilic granulocytes. During the time studied, DCs remained responsible for most of the CXCL2 production. Neutrophils began infiltrating the kidney 3 hours after the second instillation and phagocytozed bacteria. Macrophages followed 3 hours later and contributed much less to both CXCL2 production and bacterial phagocytosis. To investigate whether DCs recruit neutrophils into the kidney for antibacterial defense, we used CD11c.DTR mice allowing conditional depletion of CD11c+ dendritic cells. The absence of CD11c+ DCs markedly delayed neutrophil recruitment and bacterial clearance. In conclusion, these findings suggest that the tubulointerstitial dendritic cell network serves an innate immune sentinel function against bacterial pyelonephritis.


Bacterial pyelonephritis (PN) is the most prevalent kidney infection. Most infections are caused by uropathogenic Escherichia coli (UPEC) bearing distinct virulence factors, such as fimbriae, that facilitate attachment to uroepithelial cells, bacterial ascension from the bladder to the kidney, and organ infiltration.1,2 The innate immune defense relies on polymorphonuclear neutrophilic granulocytes (PMN),3 which are recruited by chemokines, of which CXCL2 (MIP-2) is especially important.4–6 Tubular epithelial cells can produce this chemokine, but their contribution to PMN attraction is unclear.4,5 The role of hematopoietic cells in such recruitment is unknown.

The most abundant immune cells in the kidney are CD11c+ DCs, which form an intricate network in the tubulointerstitium.8,9 Although these cells express F4/80, they functionally resemble immature DCs, and not macrophages.8 DCs are the most effective inducers of adaptive immunity. They survey nonlymphoid tissues and transport antigens to draining lymph nodes for T cell activation. Some DCs remain in tissues to regulate infiltrating effector or memory T cells,10 and this has been demonstrated also in the kidney.11–13 In addition to their well-known role in adaptive immunity, DCs also regulate innate immunity, for example, in splenic Listeria infection.14 The role of DCs in PN is unclear.

We established an experimental model of bacterial PN by transurethral instillation of E. coli from the uropathogenic strain 536 (UPEC) into the bladder of C57BL/6 mice. In previous studies, infection rates of 20 to 30% were described.1,4 Confirming these reports, we achieved PN at 24 hours after a single transurethral instillation of UPEC in 16% of the mice, as detected by determining bacterial CFU after culturing homogenates of infected kidneys on LB agar plates (Figure 1A). Such low infection rates preclude comparing groups of experimental mice. However, we discovered that a second transurethral instillation of UPEC 3 hours after the first one raised the infection rate to almost 84% (Figure 1A). Performing the second instillation after 21 hours was ineffective (data not shown). UPECs possess virulence factors that support their ascension through the ureter into the kidney,2 and these may explain why the first instillation was so permissive for contracting infection after the second one. For clarity, the time...
points given here always count from the second bacterial instillation because then most of the mice contracted PN. Dose titration identified a number of 10^9 UPECs as optimal (Figure 1B). Kinetical analysis showed that kidney CFU at 3 or 21 hours after the second bacterial instillation were similarly high, whereas they had mostly declined after 3 or 5 days (Figure 1C).

To study the immune response in PN, we determined phagocyte infiltration by quantifying the number of DCs, macrophages (MΦs), and PMN, as recently described. An analysis example is shown in Figure 2A. It was important to vigorously perfuse the kidneys before analysis to remove the large number of intravascular leukocytes that otherwise would profoundly alter the results (Figure 2B). In noninfected animals, most leukocytes were resident DCs, whereas only few MΦs and PMN were detected (Figure 2, A and C). Already 3 hours after second UPEC instillation, a strong increase in the number of PMN but no significant increase of MΦs was noted (Figure 1C).

To determine the contribution of these phagocytes to bacterial clearance, we infected mice with UPECs expressing recombinant green fluorescent protein (GFP). On a per cell basis, PMN and MΦs captured much larger amounts of UPEC-GFP than did DCs, and the proportion of PMN that had captured GFP was higher than that of MΦs at 3 hours (Figure 3A) or 21 hours (Supplemental Figure 1) after the second infection. However, the contribution of these cells to total bacterial uptake also depended on their abundance in the kidney. We had shown above that PMN and MΦs accumulated, and DC numbers declined during PN (Figure 2, A and C). Therefore we determined by flow cytometry the absolute number of GFP^+ cells in a given phagocyte subset (Supplemental Figure 2A) and the average GFP content per phagocyte (Supplemental Figure 2B). The product of these two parameters indicated the total UPEC uptake by all cells in a given phagocyte subset (Figure 3B).

Using this approach, we found that PMN had taken up 77% of the GFP at 3 hours, whereas DCs and MΦs accounted for 19 and 4%, respectively (Figure 3B). After 21 hours, PMN were responsible for almost all phagocytosis, and DCs and MΦs accounted for 3 and 7%, respectively (Figure 3B). These findings indicated that PMN were the most active phagocytes in PN, consistent with their essential role in this infection.

We next determined the cell that produced CXCL2 for PMN recruitment. CD45^+ nonimmune cells failed to produce this chemokine, whereas CD45^+ CD11c^+ DCs produced most of it (Figure 3C). Both the number of chemokine-producing DCs (Supplemental Figure 3A) and the chemokine production per DCs (Supplemental Figure 3B) were higher than those in MΦs and PMN. DCs accounted for 77%, MΦs for 13%, PMN for 10%, and other immune cells for none of the total CXCL2 at 3 hours (Figure 3D). These findings suggested that DCs recruit PMN in PN.

To determine the necessity of DCs for PMN recruitment, we employed CD11c.DTR mice expressing the diphtheria toxin (DT) receptor (DTR) and GFP under the CD11c promoter, which allow conditional DC ablation. These mice had previously been used to clarify the roles of DCs in numerous immune processes, including cross-priming and listeriosis, asthma, several types of nephritis, and many more. We planned analysis of PMN recruitment at 3 hours after second instillation (experimental scheme in Figure 4A) because then PMN numbers were increased already (Figure 2C). However, at that time point (equal to 6 hours after DC depletion) intrarenal DC numbers were only partially...

Figure 1. Repetitive instillation of UPEC increases incidence of PN. (A) Number of CFU of UPEC in homogenates of one kidney after single or double UPEC instillation. The percentages next to the brackets give the proportion of mice with detectable infection. (B) CFU per kidney at 21 hours after the second bacterial instillation as a function of the number of UPEC instilled. (C) CFU per kidney at different time points after the second instillation of 10^9 UPEC. Results are representative of 14 experiments in groups of 6 mice.
reduced (Figure 4B, right panel). Near-complete DC disappearance required 24 hours after DT injection (1.7 ± 0.5 × 10^4 cells per kidney as compared with 14.0 ± 3.4 × 10^4 in controls, n = 2 × 3 mice). Nevertheless, at the early time point, the kidney DCs were already functionally incapacitated because they did not produce CXCL2 any more (Figure 4C, Supplemental Figure 4), indicating that they had ceased to function before their death became apparent. Neither monocyte and PMN numbers in the blood nor PMN and MΦ numbers in the kidney were significantly changed by DT injection at that time point (Figure 4B), indicating specific DC depletion.

We observed that kidney CFU slightly but reproducibly differed between infected CD11c.DTR (12.5 ± 4.1 × 10^5 CFU) and infected nontransgenic C57/BL6 mice (6.9 ± 2.1 × 10^5 CFU). Therefore, we used CD11c.DTR mice not injected with DT as a control in this study. Nontransgenic mice injected with DT or not showed equal bacterial CFU (7.9 ± 2.7 × 10^5 versus 6.9 ± 2.1 × 10^5 per kidney) and PMN numbers (1.3 ± 0.2 × 10^4 versus 1.6 ± 0.3 × 10^4 cells per kidney) after 24 hours, excluding that DT injection as such affected early PN. We observed no signs of urosepsis after infecting DC-depleted mice, consistent with our previous study in bacterial cystitis.21

Having established the experimental conditions, we determined the course of infection in DC-depleted CD11c.DTR mice (experimental scheme in Figure 4A). The bacterial load in the kidney was more than sevenfold higher at 3 hours after the second instillation than in non-

Figure 2. PMN and macrophages are rapidly recruited in PN, whereas DCs are kidney-resistant. (A) Representative flow cytometry dot plots at different time points after second instillation of 10^9 UPEC into CX3CR1^+/GFP mice. Among the CD45^+ kidney cells, CX3CR1^HI Gr-1^LO F4/80^+ CD11c^+ DCs, CX3CR1^INT Gr-1^INT F4/80^+ CD11c^+ macrophages (MΦs), and CX3CR1^+ Gr-1^HI F4/80^− CD11c^− PMN were discriminated as indicated. (B) Mice were infected with 10^9 UPEC and renal DCs, MΦs, or PMN were enumerated after 24 hours in infected (black bars) and noninfected (white bars), perfused (hatched bars) and nonperfused (nonhatched bars) mice. (C) Number of DCs (■), MΦs (▲), and PMN (▼) per kidney at different time points after second instillation of 10^9 UPEC. Results are representative of three experiments in groups of five mice.
depleted controls (Figure 4D), indicating impaired bacterial clearance. Intrarenal PMN numbers were 70% decreased at 3 hours (Figure 4E), indicating DC-dependent PMN recruitment. A clear reduction of PMN recruitment was directly evident on kidney sections of infected DC-depleted mice (Figure 4F). At 21 hours after infection, PMN numbers in DC-depleted mice were still reduced (Figure 4E). This reduction was smaller than that after 3 hours, which may be explained by the stronger infection in the absence of DCs (Figure 4D), which presumably had induced other recruitment mechanisms that partially compensated for the loss of DCs, albeit with a delay. Consistent with delayed infectious control, the infectious load at 21 hours was lower than that at 3 hours (Figure 4D). Taken together, these findings demonstrated that DCs facilitated early PMN recruitment and thus innate antibacterial defense in PN.

Kidney DCs are a surprisingly abundant cell population in the tubulointerstitium, whose role in renal disease is currently emerging. DCs in the healthy kidney rapidly alerted the immune system in models of acute kidney injury and stimulated intrarenal T cell responses in chronic but not acute glomerulonephritis. Here, we demonstrate a novel function of kidney DCs, the induction of innate immunity against bacterial PN by rapid production of chemoattractants like CXCL2, which others have shown to be especially important in urinary tract infection. Without DCs, PMN recruitment and bacterial clearance were much delayed, and delays may be critical in infections with rapidly replicating pathogens. There may be DC-independent mechanisms responsible for such delayed PMN recruitment. Nonhematopoietic cells did not produce CXCL2, but may secrete other PMN-attracting chemokines like CXCL1. MΦs and PMN did produce some CXCL2 and might recruit further PMN after entering the kidney. However, MΦs did so at a later time point and are less frequent than DCs. Thus, they are less suitable as organ-resident sentinels against pathogens. By contrast, DCs are located right adjacent to the tubuli through which UPEC ascend from the renal pelvis into the organ and therefore are strategically positioned for sensing bacteria and rapidly inducing innate immunity. This sentinel function may be an important purpose of the abundant tubulointerstitial DC network.

**CONCISE METHODS**

**Mice and Reagents**

All mice were bred and kept under SPF conditions at the central animal facilities of the Bonn Medical Faculty. CD11c.DTR mice and CX3CR1GFP/GFP mice were from Steffen Jung (Rehovot). All lines had been backcrossed.
For DC depletion, CD11c.DTR mice were injected with DT (Sigma-Aldrich). Animal experiments had been approved by a local animal ethics reviewing board.

Urinary Tract Infection Model
Uropathogenic E. coli strain 536 (UPEC) and E. coli strain 536-gfp (UPEC-GFP) were

Figure 4. Depletion of DCs delays PMN recruitment and bacterial clearance in UTI. (A) Experimental scheme. (B) CD11c.DTR mice were injected with DT (hatched bars) or not (white bars), and after 6 hours, the number of PMN and monocytes (MONO) in the blood, and of PMN, MPs, and DCs in the kidney were determined by flow cytometry. (C through E) CD11c.DTR mice were injected with DT (hatched bars) and instilled with UPEC (black bars) for the first time at −3 hours and at the second time at 0 hours and analyzed at 3 hours for intracellular CXCL2 content in DCs by flow cytometry (C) and for number of CFU (D) and PMN (E) per kidney at 3 and 21 hours by flow cytometry. The results in Figure 4C were calculated from the data in Supplemental Figure 4. (F) Representative kidney sections from noninfected (upper row) or infected (lower row) CD11c.DTR mice injected with DT (right column) or not (left column). Arrows indicate individual PMN. Results are representative of three experiments in groups of five mice.

>10 times to the C57BL/6 background. For DC depletion, CD11c.DTR mice were injected with 8 ng/g body wt DT (Sigma-Aldrich). Animal experiments had been approved by a local animal ethics reviewing board.
grown for 5 hours at 37°C in LB medium so that they were in their exponential growth phase. Bacteria were harvested by centrifugation at 4600 rpm for 10 minutes and resuspended in 2 ml of LB medium to a final concentration of 1 × 10^10 CFU/ml. Female mice of 8 to 12 weeks of age were anesthetized with Avertin (40 mg of 2,2,2-tribromoethanol [Sigma-Aldrich] dissolved in 1 ml of tert-amyl alcohol [0.01 ml/g body wt intraperitoneally]) and were infected by transurethral instillation of 1 × 10^8 UPEC using a flexible polyethylene catheter (outer diameter 0.6 mm; BD) coated with Instillagel (Farco Pharma). Three hours later, the procedure was repeated to induce PN. Before analysis, mice were perfused with sterile PBS. The number of ascended bacteria was quantified by scoring CFU after overnight culture of kidney collagenase digest or by homogenization in PBS with an Ultra Turrax at 37°C on CPS ID plates (Biomerieux) as described.21

Isolation of Phagocytes from the Kidney

A previously described protocol was used.8 In brief, kidneys were sliced with a scalpel into six similar pieces and digested for 25 minutes at 37°C with 0.5 mg/ml collagenase and 100 μg/ml DNase I in RPMI 1640 medium (Invitrogen) containing 10% heat-inactivated FCS (PAA Laboratories), 20 mM Hepes, 1 mM L-glutamine, and antibiotics (Sigma-Aldrich). After digestion kidneys were mashed and incubated for an additional 15 minutes at 37°C. Single-cell suspensions were filtered through a 100-μm nylon mesh and washed with PBS lacking Ca^{2+} and Mg^{2+} but containing 0.1% heat-inactivated FCS and 0.5% sodium azide for surface marker analysis and without sodium azide for intracellular staining.

Flow Cytometry

Single-cell suspensions were incubated with 2.4G2 culture supernatant to block Fc receptors. Titrated amounts of the following labeled antibodies from Pharmingen or eBioscience were used for staining of 1 × 10^6 cell per sample: anti-CD11b-PE, -APC (M1/70), -Gr-1-PerCP-Cy5.5 (RB6-8C5), and anti-CD11c-FITC and -APC (HL-3), and Streptavidin-APC. Anti-F4/80-PE (Cl:A3–1) and anti-CD45-APC-Cy7 and -PacificBlue (30-F11) were from BioLegend. Anti-©XCL2-biotin (AAM48B) was from AbD Serotec. Dead cells were excluded with Hoechst3342 and for intracellular staining the LIFE/DEAD® fixable violet dead cell stain kit from Invitrogen was used.

For intracellular staining 1 μl/ml Golgi-Plug (BD) was used for 4 hours to block cellular release of chemokines, and PermWash (BD) was used instead of PBS. Cells were analyzed on an LSR II cytometer (BD) or a CANTO II cytometer (BD) using FlowJo software (Tristar). Forward and side scatter gating was adapted to include all leukocytes.

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