Kidney Dendritic Cells Become Pathogenic during Crescentic Glomerulonephritis with Proteinuria

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ABSTRACT

It is unclear why kidney dendritic cells attenuate some models of kidney disease but aggravate others. Kidney dendritic cells ameliorate the early phase of nonaccelerated nephrotoxic nephritis, a murine model of crescentic glomerulonephritis, but their effect on the later phase is unknown. Here, we report that kidney dendritic cells at later stages of nephrotoxic nephritis expressed higher levels of costimulatory molecules but lower levels of the cosuppressor molecule ICOS-L and started production of IL-12/23p40 and TNF-α. Furthermore, we noted that kidney dendritic cells captured more filterable antigen in proteinuric mice at late time points of nephrotoxic nephritis and started to capture molecules that were too large for filtration by a healthy kidney. They presented filtered antigen to Th cells, which responded by producing the proinflammatory cytokines IL-2, IFN-γ, TNF-α, IL-6, and IL-17. Notably, production of the suppressive cytokine IL-10 further increased in late nephrotoxic nephritis. Depletion of kidney dendritic cells at a late stage attenuated nephrotoxic nephritis, in contrast to the exacerbation observed with depletion at an early stage, indicating that their acquired proinflammatory phenotype adversely affected disease. These findings indicate that the intrarenal inflammatory microenvironment determines how kidney dendritic cells affect nephritis. In addition, proteinuria may harm the kidney by providing dendritic cells with more antigens to stimulate potentially pathogenic Th cells.


An extensive network of resident dendritic cells (DCs) populates the kidney tubulointerstitium.1–4 DCs exist in virtually all tissues where they gather antigens for transport to draining lymph nodes for T-cell activation.5,6 Immunogenic T-cell responses require that DCs themselves become activated, for example, by pathogen-associated molecular patterns,7 and upregulate costimulatory signals like CD80, CD86, or CD40. These T-cell survival signals are also known as signal 2 in T-cell activation, whereas antigen as signal 1 determines the specificity of the T cells to be activated.8 Cytokines have been referred to as signal 3 and dictate the functional differentiation of the activated T cells.9 T cells activated by immature DCs that cannot provide signals 2 and 3 become tolerized.6,10 In addition to the canonical function of T-cell activation in lymphatic organs, nonmigratory DCs that remain in tissues regulate infiltrating effector T cells6,11 or stimulate tissue-resident memory T cells.12

Despite extensive studies on DCs in various diseases, their role in renal disease is still incompletely understood. Kidney DCs (KDCs) have
been recognized as the earliest producers of proinflammatory cytokines in renal ischemia\(^3\) and experimental unilateral ureter ligation,\(^14\) suggesting a sentinel role against kidney injury. This interpretation is supported by proinflammatory responses of KDCs against immunostimulatory self-molecules in MRL-lpr mice.\(^15\) We recently showed that KDCs capture T cell–released glomerular antigens and present them to CD4\(^+\) T helper (Th) cells, resulting in production of cytokines like IL-12 and IFN-\(\gamma\) and of chemokines that maintained tubulointerstitial mononuclear infiltration,\(^11\) a hallmark of progressive kidney disease. These infiltrates were particularly pronounced around inflamed glomeruli, a feature seen also in rapid-progressive

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By contrast, KDCs were protective in other models.\(^18\) In cisplatin-induced tubular damage, their depletion exacerbated renal pathology and compromised kidney function within a few days.\(^19\) This study excluded release of proinflammatory mediators from dying KDCs after depletion as the underlying reason. Transfusion of functionally immature donor KDCs prolonged graft survival.\(^20,21\) We also showed a protective role in nephrotoxic nephritis (NTN),\(^22\) a widely studied murine model of crescentic GN.\(^23\) KDCs from nephritic mice induced in Th cells not only production of typical Th type 1 (Th1) cytokines, but also of IL-10,\(^2,2\) which can attenuate NTN.\(^23–25\) KDCs also expressed inducible costimulatory molecule ligand (ICOS-L)\(^22\) that can induce IL-10 and is protective in NTN.\(^26\) At present, it is unclear why KDCs acted pro- or anti-inflammatory in different kidney disease models.

NTN is induced by injecting sheep anti-serum against kidney cortex antigens, which is deposited in glomeruli and taken up by antigen presenting cells, such as DCs and macrophages. These stimulate Th1 cells, resulting in delayed type hypersensitivity–like kidney injury.\(^23\) Recent studies reported a contribution of Th17 responses in several nephritis models, including NTN.\(^27–29\) This is not inconsistent with previous studies proposing a role of Th1 cells,\(^30,31\) because these studies had targeted p40, the common component of IL-12 and IL-23 and thus both Th1 and Th17 responses. In addition to these cytokines, the Th1 mediator IFN-\(\gamma\), and TNF-\(\alpha\) that is produced in both Th1 and Th17 responses, promote kidney damage in NTN.\(^23,32,33\)

The nature of the antigen that KDCs present to stimulate intrarenal Th cells in NTN is unclear. In healthy mice, DCs within the kidney constitutively take up filterable antigen, such as proteins below albumin size.\(^34\) Studies examining capture of filterable antigen under conditions of proteinuria, a typical feature of NTN, are lacking. Here, we compared functional properties of KDCs at different time points of NTN. We show a hitherto unknown functional switch of KDCs with implications for disease progression.

RESULTS

KDCs Mature during the Course of NTN

To investigate whether the phenotype and activation state of KDCs changes during the course of nonaccelerated NTN, we obtained single cell suspensions from kidneys of healthy mice and nephritic mice in an early (day 4) and a late stage (day 10) of disease and analyzed them for expression of activation/maturation markers by flow-cytometry. Surface expression of CD40, CD80, and CD86, which aggravate NTN,\(^35,36\) increased with progression of NTN (Figure 1A). Differences between 4 and 10 days after disease induction reached statistical significance for CD40 and CD86 but not for CD80 expression (Figure 1A). ICOS-L, which is protective in NTN,\(^26\) was upregulated on DCs in early NTN but decreased significantly between 4 and 10 days after disease induction (Figure 1B). Consistent with our earlier study,\(^22\) we failed to detect differences in expression of the DC subset markers CD11b, CD8, and B220 (data not shown), indicating that the subset composition did not change during disease. However, we noted that the percentage of Gr1–expressing DCs increased during disease (Figure 1C), suggesting recruitment of circulating Gr1\(^+\) monocytes that differentiated into DCs. Consistent with recruitment, KDC numbers were increased approximately two-fold in nephritic mice (Figure 1D). We next analyzed production of proinflammatory cytokines known to be important in NTN via flow cytometry. The percentage of KDCs producing TNF-\(\alpha\) (Figure 2A) and IL-12/23p40 (Figure 2B) increased after NTN induction in a time-dependent manner. CD11c-negative immune cells produced these cytokines neither on day 4 (data not shown) nor on day 10 (Figure 2, C and D). It has to be kept in mind, however, that in this experiment, \(\text{ex vivo}\) cytokine production without re-stimulation was examined. T cells require such stimulation; hence, this experimental setting does not permit conclusions on the contribution of T cells.

To determine whether these cytokines were produced by recently recruited DCs, we costained KDCs for Gr1 and TNF-\(\alpha\) or IL-12/23p40. Flow cytometric analysis showed that Gr1–DCs were responsible for most of the cytokine production, whereas Gr1\(^+\) DCs contributed only small amounts (Figure 2, E and F). These results indicated that KDCs acquire full immunogenic qualities at later stages of NTN.

KDCs Capture More Filterable Antigen in Late NTN

The findings above established that KDCs provide more of the signals 2 (costimulation) and 3 (cytokines) for T-cell activation in late NTN. We next asked whether they could also provide more signal 1 (antigen). To this end, we took advantage of our recent finding that KDCs efficiently capture low-molecular-weight antigens that are constitutively filtered in glomeruli, such as ovalbumin (OVA; 45 kD).\(^34\) Proteinuria increased with progression of disease (albumin excretion per 16 hours: healthy mice \(=\text{day 0}\): 0.1 ± 0.05 mg; day 4: 397.2 ± 152.5 mg; day 10: 1164 ± 605.7 mg). To test whether proteinuria in NTN affects capture and presentation of filterable molecules, we in-
jected healthy and nephritic mice at an early (day 4) and a late (day 10) time point with 500 ng fluorochrome-labeled OVA per gram body weight. After 30 minutes, we obtained single cell suspensions from the kidneys and analyzed antigen uptake by KDCs via flow cytometry (Figure 3, A, C, and D). In early NTN on day 4, the proportion of KDCs that took up filterable OVA was similar to healthy mice (Figure 1D). However, their absolute numbers were higher, because total DC numbers were increased on that day (Figure 1D). Furthermore, those KDCs that captured OVA on day 4 took up more of it (Figure 3D). In late NTN on day 10, OVA was internalized by a higher percentage of KDCs (Figure 3C) and at greater amounts per KDC (Figure 3D) compared with early NTN and healthy mice. These findings showed that KDCs took up more filterable antigens in late NTN.

In a healthy kidney, antigens above albumin size are usually not filtrated. To test whether the proteinuria that occurs during NTN allows KDCs to acquire such antigen, we injected healthy and nephritic mice with 10^6 fluorochrome-labeled 500-kD dextrane per gram body weight and analyzed antigen uptake after 60 minutes by flow cytometry. This large antigen was taken up by >20% of KDCs in healthy mice (Figure 3, B, E, F).
and F), perhaps from peritubular capillaries. However, in NTN, increasing percentages of KDCs took up increasing amounts of 500-kD dextrane, indicating that, under proteinuric conditions, KDCs capture not only filterable antigen but also large antigens that normally are not filtered (Figure 3, B, E, and F).

Importantly, antigen uptake strongly correlated with surface expression of CD80 and CD86 (Figure 3G), indicating that those KDCs that provide signal 1 simultaneously provide signal 2. The correlation between antigen uptake and expression of CD40 and ICOS-L was less pronounced (Figure 3G).

Figure 2. Cytokine production by KDCs increases during the course of NTN. (A and B) Percentage of KDCs producing TNF-α (A) or IL-12/23p40 (B) in healthy 4- and 10-day nephritic mice, as determined by intracellular cytokine staining and flow cytometry. Results are representative for two separate experiments. (n = 3 to 4 mice per group; **P < 0.01). (C and D) Representative dot plots of kidney CD45+ cells from 4-day nephritic mice showing TNF-α (C) and IL-12/23p40 (D) production as a function of CD11c expression. (E and F) Representative dot plots of kidney CD11c+ cells from 4-day nephritic mice showing TNF-α (E) and IL-12/23p40 (F) production as a function of Gr1 expression.
Figure 3. KDCs capture more filterable antigen in late NTN. (A) Mice were intravenously injected with 500 ng fluorescently labeled OVA (45 kD) per gram body weight on day 0, 4, or 10 after NTN induction. Thirty minutes later, single cell suspensions gated for CD45⁺ cells were analyzed for OVA uptake via flow cytometry. (B) The same experiment as shown in A, except that mice received 10 μg fluorescence-labeled 500-kD dextrane per gram body weight instead of OVA and that cells were analyzed after 60 minutes. (C) Percentage of CD45⁺ CD11c⁺ MHCII⁺ KDCs containing OVA in kidney single cell suspensions from healthy (day 0), early-nephritic (day 4), and late-nephritic (day 10) mice 30 minutes after intravenous injection of OVA (n = 3). (D) Mean fluorescence intensity (MFI) of OVA⁺ KDCs, indicating the amount of antigen taken up per DC (n = 3). (E and F) Percentage (E) and MFI (F) of CD45⁺ CD11c⁺ MHCII⁺ KDCs positive for 500-kD dextrane 1 hour after intravenous injection (n = 3 to 4). (G) Mice were intravenously injected with 500 ng fluorescence-labeled OVA (45 kD) per gram body weight on day 10 after NTN induction, and expression of CD40, CD80, CD86, and ICOS-L was determined. Representative contour plots show the expression of costimulatory molecules as a function of antigen uptake. *P < 0.05; **P < 0.01.
KDCs Use Filtrated Antigen to Stimulate Th1 and Th17 Responses More Effectively in Late NTN

To study consequences for Th-cell activation, we isolated KDCs from healthy or nephritic mice injected with OVA and cultured them alone or with OVA-specific CD4+ T cells (OT-II cells). As an additional control for antigen-unspecific T-cell stimulation, KDCs from 10-day nephritic mice, which had not received OVA, were cocultured with OT-II cells (dashed lines). KDCs from healthy mice only stimulated production of small amounts of the T-cell growth factor IL-2 (Figure 4A) and of the NTN-driving cytokines TNF-α (Figure 4B), IFN-γ (Figure 4C), and IL-17 (Figure 4D), as well as IL-6 (Figure 4E). KDCs from day 4 nephritic mice did not induce appreciably more of these cytokines, with the exception of IL-17 (Figure 4, A–E). However, KDCs from 10-day nephritic mice stimulated production of all proinflammatory cytokines very potently. Remarkably, production of the suppressive cytokine IL-10 also continued to increase during disease and was strongest in cocultures with KDCs from day 10 nephritic mice, with the majority being produced by DCs (Figure 4F). However, IL-10 levels increased very little over background (cells without antigen), and differences were not statistically significant, arguing against a role of IL-10 in KDC-dependent regulation of NTN. These findings showed that KDCs increase their ability to induce proinflammatory cytokines in late NTN.

Late DC Depletion Attenuates NTN

If the ability of KDCs to provide more of the signals 1, 2, and 3 in late NTN was functionally relevant for progression of disease, their depletion at late disease stages should be protective rather than harmful. To test this conjecture, we induced NTN in CD11c-DTR mice (DTR)37 and depleted DCs after 7 days by injection of 4 ng diphtheria toxin per gram body weight, thereby reducing KDC numbers by approximately 66% (Figure 5A). In our earlier study, we established that DC depletion by this technique in early NTN did not directly affect renal macrophages.22 DC depletion on day 7 after disease induction only transiently reduced total numbers of macrophages and did not directly alter numbers of Th cells (Figure 5B). However, 3 days after DC depletion, analysis of disease severity showed that T-cell numbers were significantly reduced by 65% in the tubulointerstitium and by 70% in glomeruli (not statistically significant) in DC-depleted mice compared with control mice, whereas numbers of tubulointerstitial and glomerular macrophages were reduced by 40%, as determined by histologic staining of CD3+ or MAC-2+ cells in kidney sections, respectively (Figure 5, C–F). Albuminuria (normalized to creatinine excretion) was slightly reduced, albeit not statistically significantly (Figure 5G). Creatinine clearance was not altered in this setting (data not shown). Histologic analysis on day 10 after disease induction showed a significant reduction

![Figure 4](https://www.jasn.org/content/22/2/306.full)

Figure 4. KDCs activate Th cells specific for filtrated antigen more effectively. KDCs (10^5) from healthy (0 days), 4-day, and 10-day nephritic mice, which had been injected with 700 μg of OVA 1 hour before DC isolation, were cultured alone (DC-culture: white bars) or cocultured with 2 × 10^5 OT-II cells (Coculture: black bars) for 24 hours in 250 μl RPMI (10% FCS). As a control for antigen specificity, KDCs from 10-day nephritic mice that had not received OVA were cocultured with OT-II cells (dashed lines). Concentrations of IL-2 (A), TNF-α (B), IFN-γ (C), IL-17 (D), IL-6 (E), and IL-10 (F) in the supernatants were measured by Th1/Th2 multiplex bead assay. Results are representative for three separate experiments (n = 3). **P < 0.01; ***P < 0.001.
in crescent numbers in diphtheria toxin–injected DTR mice compared with diphtheria toxin–injected nontransgenic littermates (Figures 5H and 6). Thus, late DC depletion reduced renal infiltrates by macrophages and T cells, the most relevant effector cells of NTN, as well as crescents, indicating that KDCs aggravated late NTN.

DISCUSSION

It is unclear why KDCs attenuate some models of kidney disease \(^{19,22}\) and aggravate others. \(^{11,13,14}\) In nonaccelerated NTN, we previously depleted DCs on days 4 and 10 after disease induction and noted more severe renal damage on day 12,\(^{22}\) indicating a protective role for KDCs in NTN. Here, we report that KDCs aggravate disease on day 7 after induction of NTN, indicating a functional switch from protective to exacerbating functionality between days 4 and 7. This implies that the second DC depletion we performed in our previous study on day 10\(^{22}\) evidently had been unable to repair the damage that had occurred between days 4 and 7 in that study. It is conceivable that KDCs are even more protective before day 4 in NTN, but this cannot be experimentally tested, because this model requires DC-mediated priming of T cells against sheep Ig. Because such priming usually requires approximately 3 days,\(^{22}\) earlier DC depletion would thwart disease development.

In the nephrin-OVA/HEL (NOH) model of T cell–mediated glomerular injury, we previously observed a role of KDCs similar to that in day 7 nephritic mice reported here: depletion of KDCs on day 5.5 disbanded established tubulointerstitial and periglomerular infiltration and attenuated disease.\(^{11}\) One major difference between NTN and the NOH model is that the latter involves injection of Th cells that had been activated already, whereas in NTN, these cells need to be activated in vivo, which requires about 3 days,\(^{22}\) as mentioned above. Hence, day 5.5 after injection of activated Th cells into NOH mice probably corresponds to day 8 or day 9 after NTN induction. Thus, the timeline of acquisition of proinflammatory functionality in both models is coherent. The purpose for which KDCs undergo this functional switch is speculative. Perhaps it is important to support T-cell responses in infections. T cells normally follow innate immunity after several days, corresponding to the time when KDCs had acquired T cell–stimulatory properties in NTN.

Consistent with acquisition of proinflammatory functionality, KDCs upregulated costimulatory molecules like CD80 or CD86, both of which are essential in NTN,\(^{36}\) and produced proinflammatory cytokines that promote NTN, such as TNF-\(\alpha\) and IL-12/23p40,\(^{30,31,33,38}\) Simultaneously, expression of the suppressive signal ICOS-L decreased. Consequently, KDCs isolated from nephritic mice stimulated Th cells with increasing effectiveness during the course of disease and caused production of proinflammatory cytokines that can
drive disease progression, namely IFN-γ, TNF-α, IL-17, IL-2, and IL-6. Although the latter two mediators have not yet been explicitly implicated in NTN, it is very likely that both also promote renal inflammation.

IL-10 attenuated most, although not all, experimental nephritis studies, and we expected it to be protective also in early NTN. To our surprise, the ability of KDCs to produce IL-10 further increased after day 4, and the IL-10 production by Th cells was comparatively small. Although these findings did not support a causative role of IL-10 in disease attenuation on day 4, they did not formally exclude it. Nevertheless, they did allow concluding that disease aggravation in late NTN did not result from reduced IL-10 production. Most likely, the proinflammatory signals that KDCs provided in late NTN prevailed and drove a nephritogenic Th cell response.

KDCs induced also the main Th17 cytokine IL-17 in our coculture experiments. Th17 responses have recently been implicated in NTN, and it is currently unclear whether and to which extent Th1 and Th17 are required for NTN. Interestingly, IL-17 was induced by KDCs from day 4 nephritic mice, as opposed to IFN-γ, suggesting that Th17 responses may operate at earlier time points in NTN than Th1 responses. Future studies are warranted to address this possibility.

This functional switch of KDCs might partially be caused by kidney infiltration by proinflammatory DCs recruited from the circulation, because we noted that some KDCs expressed Gr1, again reminiscent of observations in NOH mice. This marker indicates derivation from proinflammatory monocytes, the precursors of proinflammatory DCs, and is gradually lost after entering the kidney or other tissues. However, only 7 to 11% of KDCs were Gr1, whereas maturation markers were increased on nearly all DCs, indicating that resident KDCs were also activated during the course of NTN. In support of this, the KDCs expressing Gr1 showed less cytokine production than resident DCs. The latter finding also suggests that infiltrating monocytes may require some time to acquire proinflammatory functions. The exact contribution of local DC maturation and recruitment of proinflammatory monocyte-derived DCs to the acquisition of nephritis-driving functionality at present cannot be clarified because of lack of techniques to selectively target resident or recruited KDCs.

An open question in crescentic GN concerns the identity of the antigens presented by intrarenal DCs and macrophages to infiltrating Th cells. Our study showed that KDCs, especially those expressing more costimulatory molecules, acquire far more filtrated antigen in NTN, which likely is a consequence of its higher availability in the glomerular filtrate under conditions of proteinuria. Compared with days 0 and 4, more KDCs captured higher antigen amounts on day 10, which may be because of recruitment of additional DCs to intrarenal sites where filtrated antigen is available. Furthermore, KDCs also captured an antigen too large to be filtered in a healthy glomerulus. Such antigens may include food components, circulating antigens from pathogens during infections, or immune complexes. Hence, KDCs not only increase their expression of costimulatory signals (signal 2 in T-cell activation), and produc-
tion of differentiation-driving cytokines, also known as signal 3,\(^9\) but also the quality and quantity of “signal 1,” the antigen that is presented. Intensifying all three signals synergistically enhanced their ability to drive Th1 and Th17 responses. These findings suggest that proteinuria can harm the kidney by stimulating nephritogenic adaptive immune responses, in addition to previously observed toxic effects on tubular epithelial cells with subsequent activation of the innate immune system.\(^{45,46}\)

In summary, we reported that KDCs can use filterable antigens for driving intrarenal Th1 and Th17 responses, especially in proteinuria, when they acquire more filterable antigen and antigens too large to be filtered under homeostatic conditions. Furthermore, during the course of nephritis, KDCs become immunogenic and drive rather than inhibit harmful Th responses, because they provide more of the signals 1, 2, and 3 to Th cells. Distinct functionality in early and late nephritis can reconcile our previous observations that KDCs attenuated NTN22 but aggravated the NOH model.\(^3\) Our findings may also have therapeutical implications: KDCs in patients with chronic glomerulonephritis will likely have switched to a proinflammatory state already, and targeting these cells may be beneficial. By contrast, in acute glomerulonephritis or in an acute relapse of glomerulonephritis, KDCs may act in a protective manner until they have matured.

### CONCISE METHODS

**Mice, Reagents, and Nephrotic Nephritis Model**

Eight- to 10-week-old C57/BL6, CD11c-DTR/GFP,\(^3\) and OT-II mice were bred and kept under specific pathogen-free conditions at the animal facility of the University of Bonn. Nephrotic sheep serum was generated as described previously.\(^47\) The amount of nephrotic sheep serum used in this study was 1.5 mg Ig per gram body weight, which led to high proteinuria but not to fatal renal failure (data not shown). DC depletion was performed by intraperitoneal injection of 4 ng diphtheria toxin per gram body weight. Institutional and Government Review Boards approved all animal studies. All reagents were purchased from Sigma-Aldrich (Steinheim, Germany), if not specified otherwise.

**Histology**

Light microscopy was performed on 3-μm paraffin sections of paraformaldehyde-fixed tissue stained by periodic acid-Schiff. Kidney damage was histologically determined by an observer blinded to the identity of samples as described previously.\(^11,27,28\)

**Isolation of Murine Kidney DCs and OT-II Cells**

Kidneys were digested with collagenase (Roche Diagnostic, Mannheim, Germany) and DNase-I as described previously.\(^2\) Tubular fragments from digested kidneys were removed by filtration. CD11c\(^+\) DCs were enriched using nanobead-labeled CD11c-specific monoclonal antibodies (clone N418; Miltenyi, Bergisch-Gladbach, Germany). Magnetic bead separation was done according to manufacturer’s instructions. Purity was usually 90 to 95%. OT-II cells were isolated from spleen and lymph nodes of OT-II mice using nanobead-labeled Cd4-specific monoclonal antibodies (clone GK1.5). Purity was usually about 85%.

**Flow Cytometry**

After treatment with block serum (50% HBSS, 50% PBS, 1 mM EDTA, 1% FCS, 1% mouse serum, 1% rabbit serum, 1% human serum), cells were stained for 15 minutes on ice with fluorochrome-labeled monoclonal antibodies against CD45 (clone 30-F11), CD11c (HL3), I-A\(^\beta\) (AF6–120.1), CD11b (M1/70), Gr1 (RB6–8C5), CD4 (GK1.5), CD3 (145–2C11), CD40 (3/23), CD86 (GL1), CD80 (16–10A1), and ICOS-L (IIK5.3) (Ebiosciences, Kranenburg, Germany). Dead cells were excluded with Hoechst 33258, and cells were analyzed with a BD CantoII (Becton Dickinson).

**Intracellular Cytokine Staining**

Single cell suspensions were incubated in RPMI (10% FCS) with 1 μl/ml GolgiPlug (BD Biosciences) for 4 hours at 37°C. After cell surface staining, cells were fixated in 2% paraformaldehyde for 7 minutes on ice. Cell membranes were permeabilized with Saponin buffer (PBS with 2% BSA and 0.5% Saponin) for 20 minutes at room temperature. Intracellular staining was performed in Saponin buffer for 20 minutes at room temperature using fluorochrome-labeled monoclonal antibodies against TNF-α (clone MP6-XT22) and IL-12/23p40 (C17.8).

**In Vitro Cytokine Production**

For determining cytokine induction by KDC in CD4 T cells, mice were injected with 700 μg OVA. After 1 hour, KDCs were isolated. DCs (1 × 10\(^5\)) were cocultured with 2 × 10\(^5\) OT-II cells in 250 μl RPMI medium (10% FCS) in 96-well plates for 24 hours. Cytokines in the supernatants were measured applying commercial FlowCytomix kits (Bender MedSystems, Vienna, Austria).

**In Vivo Antigen Uptake**

For determining uptake of filterable and nonfilterable antigen by KDCs, mice were injected with 500 ng fluorescently labeled OVA per gram body weight or 10 μg fluorescently labeled 500-kD dextrane per gram body weight, respectively. Single cell suspensions were obtained from kidneys 30 minutes or 1 hour after antigen injection. Cells were stained and analyzed via flow cytometry.

**Miscellaneous Assays**

For determination of albumin excretion and creatinine clearance, urine was collected over 16 to 24 hours. Albumin concentrations in the urine were measured using a commercial mouse albumin ELISA kit according to manufacturer’s instructions (Bethyl Laboratories). Serum and urine creatinine concentrations were analyzed in the central laboratory of the university hospital of Bonn.

**Statistical Analysis**

Results are expressed as mean ± SEM. Comparisons were drawn using a two-tailed t test or a one-way ANOVA in combination with a Tukey post-test comparing all pairs of columns (Prism 4; Graphpad Software, San Diego, CA) except for analysis of histology, where the two-sided nonparametric Mann-Whitney U test was used.
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DISCLOSURES

None.

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