Renal Dendritic Cells Stimulate IL-10 Production and Attenuate Nephrototoxic Nephritis

Juliane Scholz,* Veronika Lukacs-Kornek,* Daniel R. Engel,* Sabine Specht,† Eva Kiss,‡ Frank Eitner,§ Jürgen Floege,§ Herrmann-Josef Groene,‡ and Christian Kurts*

*Institute for Molecular Medicine and Experimental Immunology and †Institute for Medical Microbiology, Immunologie and Parasitology, University of Bonn, Bonn, Germany; ‡Department of Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Heidelberg, Germany; and §Division of Nephrology and Clinical Immunology, RWTH University of Aachen, Aachen, Germany

ABSTRACT

The role of renal dendritic cells (DCs) in glomerulonephritis is unknown. This question was addressed in nephrototoxic nephritis, a murine model of human necrotizing glomerulonephritis, which is dependent on CD4+ Th1 cells and macrophages. DCs in nephritic kidneys showed signs of activation, accumulated in the tubulo-interstitium, and infiltrated the periglomerular space surrounding inflamed glomeruli. In ex vivo coculture experiments with antigen-specific CD4+ T cells, DCs stimulated the secretion of IL-10, which is known to attenuate nephrotoxic nephritis, and the Th1 cytokine IFN-γ. Endogenous renal CD4+ T cells produced both of these cytokines as well, but those from nephritic mice secreted increased amounts of IL-10. Renal DCs were found to express ICOS-L, an inducer of IL-10. To evaluate the in vivo role of renal DCs in disease, CD11c+ DCs were depleted on days 4 and 10 after the induction of nephritis by injecting CD11c-DTR/GFP mice with diphtheria toxin. Sparing DCs until day 4 did not affect the autologous phase of nephritis. The number of renal DCs was reduced by 70% to 80%, the number of renal macrophages was unchanged, and periglomerular infiltrates were eliminated. On days 11 to 14, we observed aggravated tubulointerstitial and glomerular damage, reduced creatinine clearance, and increased proteinuria. These findings demonstrate that renal DCs exert a renoprotective effect in nephrotropic nephritis, possibly by expressing ICOS-L and/or by inducing IL-10 in infiltrating CD4+ Th1 cells.


The murine model of nephrotropic nephritis (NTN) allows elucidating the immune mechanisms in rapidly progressive crescentic glomerulonephritis (GN). It can be induced by injection of sheep antiserum raised against kidney cortex antigens. These antibodies are deposited in the glomeruli and stimulate as antigens CD4+ Th1 cells, which then cause delayed-type hypersensitivity (DTH)-like kidney injury.1–3 Macrophages are essential effectors in this response,1,4,5 as confirmed by ablation of nephritis after conditional depletion of CD11b+ cells, which include macrophages.6 Because CD11b is also expressed by conventional dendritic cells (DCs),7,8 theoretically DCs might have been depleted as well in these studies.

Kidney DCs have been studied less extensively than macrophages, although they represent an abundant immune cell population.8,9 They are characterized by dendritic morphology10 and by expression of CD11c, CD11b, F4/80, and MHC II.8 Their MHC II expression might allow kidney DCs to interact with infiltrating CD4+ T cells and to modify their effects in NTN. In support of this notion, kidney DCs have recently been described in renal ischemia/reperfusion damage as main producers of early TNFα,11 which is known to
affect NTN. Formal experimental evidence for a role of DCs in NTN has not been reported yet. As kidney DCs are restricted to the tubulo-interstitium, a hypothetical influence on GN might affect spreading of glomerular injury to the tubulo-interstitium, which is thought to critically determine kidney function. This notion has been endorsed by DC accumulation and relocation around, but not within, inflamed glomeruli in NTN.

DCs collect tissue antigens and transport these into draining LN for T cell activation. Immunogenic activation requires prior activation of the DCs itself, for example, by pathogen-associated molecular patterns. Nonactivated, immature DCs usually tolerate T cells. Accordingly, immature kidney DCs suppressed T cell allo-reactivity. In addition to this traditional function as inducers of adaptive immunity in secondary lymphatic organs, nonmigratory DCs modify responses of activated T cells infiltrating peripheral organs. This occurs also in immunemediated diseases, as demonstrated in asthma models, where pulmonary DCs captured airborne allergens and ameliorated disease by inducing regulatory T cells (Treg) of the Tr1 type. Tr1 cells suppress other T cells by secreting IL-10 and thus differ from CD25+ “natural” CD4+ Treg (nTreg) that arise in the thymus and require direct cellular interactions, rather than cytokines, to suppress T cells. Tr1 cells in this disease model has not yet been reported, but is likely, given that many studies confirmed the beneficial effects of IL-10 on immune-mediated kidney disease. For example, IL-10 injection into rodents with NTN, lupus nephritis, or anti-Thy1.1-nephritis attenuated kidney damage, albeit some studies found no effect of this cytokine or of its inhibition, and an earlier study in NZB/W mice, which showed IL-10-dependent aggravation. Macrophages or adenoviruses genetically engineered to produce IL-10 ameliorated experimental GN, and so did hepatic IL-10 overexpression via gene transfer. Genetic IL-10 deficiency aggravated NTN, indicating a role of intrinsic IL-10, although its cellular source remained unresolved. Also, the role of DCs in kidney disease, especially in NTN, awaits clarification. In the present study, we have addressed these questions and demonstrate a novel protective mechanism operative in NTN.

RESULTS

Activated Conventional DCs Accumulate in Kidneys of Mice With NTN

We have recently demonstrated that kidney DCs accumulated and relocated to inflamed glomeruli in NTN. To further characterize these DCs, we analyzed kidney single-cell suspensions from healthy and nephritic mice by flow cytometry. On day 7 after injection of nephrotoxic sheep serum (NSS), histologic and functional signs of kidney damage were evident in a dose-dependent fashion. At this time point, DCs from nephritic...
Kidneys were increased in numbers and showed the CD11c+CD11b+ phenotype of conventional DCs (Figure 1, A and B) that DCs from healthy kidneys displayed as well (Figure 1A).8,9 Costaining for the subtype marker CD8+ showed its expression on some DCs in NTN (Figure 1A). The cells expressing high levels of CD8α and little or no CD11c (Figure 1A) lacked MHC II expression (data not shown), and thus represented CD8+ T cells. CD11c+ B220+ plasmacytoid DCs were not evident, whereas some CD11c+ B220- B lymphocytes were detected (Figure 1A). DCs from nephritic kidneys showed a more mature phenotype than those in healthy organs, as evidenced by higher expression of MHC II and costimulatory molecules such as CD80 (Figure 1C). Nephritic kidneys contained an additional CD11c+ DCs subset devoid of surface MHC II expression (Figure 1A), which represented immature DC precursors recently recruited from the blood as previously reported.8 Thus, conventional DCs of the CD8α+ CD11c+ subtype accumulated in NTN and exhibited signs of activation.

**Kidney DCs From Nephritic Mice Induce IFNγ and IL-10 Secretion**

We speculated that MHC II+ renal DCs might be able to affect the course of NTN by interacting with infiltrating CD4+ T cells, which are crucial effectors in NTN.1-3 To address this hypothesis, we isolated renal DCs from nephritic mice, allowed them to endocytose a model antigen, ovalbumin (OVA), and subsequently co-cultured them with OVA-specific CD4 T cells (OT-II cells). After 3 d, OT-II cell numbers had increased over those in cultures without antigen, indicating antigen-specific proliferation (Figure 2A). Kidney DCs from nephritic mice stimulated OT-II cell pro-

---

**Figure 2.** DCs from nephritic kidneys activate CD4+ T cells and induce IFNγ and IL-10 production. DCs from the kidneys (left graphs) or spleens (right graphs) of nephritic mice (black bars) or non-nephritic controls (white bars) were isolated, cultured with 1 mg/ml OVA or without OVA (without OVA) for 2 h, washed, and were subsequently co-incubated with 1 × 10⁶ OT-II cells. After 3 d, the number of viable OT-II cells was determined by flow cytometry (A), and the concentrations of IFNγ (B) and IL-10 (C) in the culture supernatants by ELISA. Shown are results typical of 4 independent experiments in groups of 3 or 4 mice. n.s, not significant (P ≥ 0.05); *P < 0.05.
liferation more efficiently than DCs from healthy mice (Figure 2A), demonstrating functional consequences of DC activation in NTN (Figure 1C). As expected, splenic DCs used as controls for competent DCs elicited a stronger proliferative response (Figure 2A). This response was identical in nephritic and control mice (Figure 2A), demonstrating that CD4 T cell activation in NTN was kidney-specific.

We next examined the cytokines produced in these cocultures. DCs from nephritic kidneys induced fivefold increased levels of the Th1 cytokine IFNγ, which nevertheless were 10-fold lower than those accomplished by splenic DCs (Figure 2B). Strikingly, kidney DCs from nephritic mice induced fivefold increased amounts of IL-10 (Figure 2C). Splenic DCs from healthy and nephritic mice produced little of this cytokine indicating disease- and organ-specific IL-10 induction. No cytokine production was detected when DCs or T cells were cultured separately or without antigen (Figure 2, B and C). The requirement of antigen-specific T cell stimulation for IL-10 production implied that DCs did not secrete noticeable amounts of this cytokine themselves. Thus, DCs induced not only a typical Th1 cytokine, but also the prototypic immunosuppressive cytokine, IL-10, which has been shown to suppress NTN.24–28,30–32

**Endogenous T Cells From Nephritic Mice Produce IL-10**

To investigate whether endogenous T cells also produced IL-10 in the kidney, we isolated these cells on day 5 after NTN induction. Nephritic kidneys contained more CD4+ T cells than kidney from non-nephritic controls (Figure 3A), consistent with their recruitment in NTN.8 When we stimulated equal numbers of renal T cells with anti-CD3 antibody in vitro and determined cytokine concentrations in the supernatant after 2 d, T cells from nephritic mice showed pronounced IL-10 production, whereas those from healthy mice produced 60% less of this cytokine (Figure 3B). IFNγ was produced in similar amounts regardless of nephritis (Figure 3B). Without anti-CD3 stimulation, no cytokine release was observed, indicating production by T cells (Figure 3B). These findings demonstrate that kidney T cells in NTN had been programmed for high IL-10 production on a per cell basis, whereas their ability to produce IFNγ was not altered.

**Establishing and Characterization of an In Vivo Depletion System for Kidney DCs**

Theoretically, the above cytokine profile might lead to either Th1-mediated aggravation or IL-10-mediated attenuation of NTN. To distinguish these possibilities, we established an experimental system to study the course of NTN in the absence of kidney DCs. We used transgenic CD11c-DTR/GFP mice34 expressing the human diphtheria toxin receptor in CD11c DCs and green fluorescence protein (GFP). Injection of diphtheria toxin (DT) into these animals causes systemic depletion of CD11c DCs, which can be monitored by analysis for the loss of cells expressing GFP. Without DT injection, these mice did not show any detectable immunologic alterations to wild-type C57/BL6 control mice.34 In addition, we did not observe histologic or functional differences when NTN was induced in nondepleted CD11c-DTR/GFP compared with nontransgenic control mice (data not shown).

As we intended to target kidney DCs in the effector phase of NTN, we designed a protocol that avoided effects of DC depletion on the autologous immune response against sheep immunoglobulin (Ig), while at the same time ensured equal glomerular deposition of sheep Ig in DC-depleted and nondepleted animals. This was achieved by injecting DT on day 4 after NSS injection; thus, the DC-dependent induction phase of the autologous response
Figure 4. Efficiency and specificity of DC depletion in the kidney. (A) Experimental protocol to determine specificity of DC depletion. Four days after induction of NTN in CD11c-DTR/GFP mice (black columns) or wild-type controls (white columns), all mice were injected intraperitoneally with 4 ng DT per g body weight. (B and C) On day 5, kidney (B) or spleen (C) single-cell suspensions were analyzed by flow cytometry for total numbers of CD11c⁺ DCs, CD11c⁺ CD11b⁺ macrophages, CD4⁺ and CD8⁺ T cells, and for NK cells per organ. (D) Protocol to determine the effect of DC depletion on NTN. Four and 10 days after induction of NTN in CD11c-DTR/GFP mice (black columns) or wild-type controls (white columns), mice received 4 ng DT per g body weight. (E) On day 14, kidney single-cell suspensions were analyzed by flow cytometry for CD11c⁺ cells, GFP⁺ cells, and for co-expression of CD11b and MHC II. Contour plots show representative expression profiles of these markers. (F and G) Statistical analysis of the total numbers of intrarenal CD11c⁺ DCs, CD11c⁺ DCs expressing transgenic GFP, MHC II⁺ DCs, and CD11c⁺ CD11b⁺ macrophages (F), and of CD4⁺ T cells, CD8⁺ T cells and CD19⁺ B cells (G) on day 14. (H) Numbers of splenic CD11c⁺ DCs, CD4⁺ T cells on day 14. These results are representative for 2 individual experiments using groups of 4 mice. n.s, not significant (P ≥ 0.05); *P < 0.05; **P < 0.01.
and after glomerular sheep Ig deposition (Experimental protocol in Figure 4A). This resulted in 80% loss of CD11c<sup>+</sup> DCs (n = 4 mice/group, P < 0.05), when kidneys were analyzed on day 5 (Figure 4B). Depletion was specific for DCs because intrarenal macrophages and lymphocytes (NK, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells) were not significantly changed (Figure 4B). In the spleen, DCs were reduced by 75% and macrophages by 33% (Figure 4C), consistent with previous reports showing that in the spleen of CD11c-DTR/GFP mice only DC and marginal zone macrophages were depleted.34–36 A single DT injection was unlikely to continuously deplete DCs throughout the experiment because DCs in lymphatic organs were replenished within 2 to 4 d.34 However, a second DC depletion after this short time was lethal for CD11c-DTR/GFP mice, as reported by others.37 Nevertheless, we found that a second depletion after 1 wk was tolerated and resulted in 85% reduction of kidney DCs on day 14 (Experimental protocol in Figure 4D, results in Figure 4, E and F). Numbers of DCs in the spleen were unaltered on day 14 (Figure 4H), as opposed to their efficient depletion on day 5 (Figure 4B). Thus, replenishment of DCs in the kidney occurred slower than in the spleen, resulting in prolonged absence of renal DC.

Numbers of renal macrophages on day 14 were unchanged (Figure 4, E, lower row, and F), again demonstrating that the depletion protocol specifically ablated renal DCs. Renal CD4<sup>+</sup> T cells were 40% to 50% fewer, but this was not statistically significant (Figure 4G). Renal CD8<sup>+</sup> T cells were significantly reduced by 60% (Figure 4G). However, reduced CD8<sup>+</sup> T cell numbers are unlikely to aggravate murine NTN, as this model has been shown to be independent of these cells.38 A slight nonsignificant change was seen also in numbers of renal B cells, which were examined as a control-lymphocyte population (Figure 4G). Numbers of splenic CD4<sup>+</sup> (Figure 4H) and CD8<sup>+</sup> T cells (data not shown) were unaltered, indicating that the tendency toward reduced renal T cell numbers was a secondary effect of DC depletion, consistent with reports showing that T cells are not directly targeted in CD11c-DTR/GFP mice.34,36

The DC reduction was also apparent when the MHC II<sup>+</sup> DCs were enumerated (Figure 4F), implying that less DCs remained available for interaction with infiltrating CD4<sup>+</sup> T cells. This was confirmed by immunohistochemistry, which showed a marked

Figure 5. Histologic images. Kidney sections from DT-injected CD11c-DTR/GFP mice (B, D, F, H) and controls (A, C, E, G) from the protocol shown in Figure 4D (using 4 mg sheep Ig/g body weight) were taken on day 11 and stained for MHC II (A-D) or by PAS (E-F). A, B, E, and F: original magnification ×100; C, D, G, and H: original magnification ×200. Shown are representative sections illustrating the quantitative analyses shown in Figure 7, A through D.

Figure 6. Systemic immunity against sheep Ig is not affected by DC depletion. (A) Serum samples from DT-injected CD11c-DTR/GFP mice (black bars) and controls (white bars) from the experimental protocol shown in Figure 4D were taken on day 14 and analyzed by ELISA for titers of total murine IgG, IgG1, and IgG2a levels specific for sheep IgG (mouse IgG/IgG1/IgG2a α sheep IgG). (B) 2 × 10<sup>5</sup> splenocytes from the same mice were cocultured with 10 μg/ml sheep Ig. After 3 d, concentrations of IL-10 and TNFα and, in separate experiments, of IL-2 and IFNγ, in the culture supernatant were determined by ELISA. n.s, not significant.
reduction of MHC II⁺ cells in kidneys of DT-injected CD11c-DTR/GFP mice, whereas many such cells were visible in the tubulo-interstitium of DT-injected wild-type mice (Figure 5, A and B).

In particular, the periglomerular infiltration evident in nephritic lo-interstitium of DT-injected wild-type mice (Figure 5, A and B). DTR/GFP mice, whereas many such cells were visible in the tubulo-interstitium of the murine kidney is a specific DC marker.8

Figure 7. Depletion of DCs aggravates kidney damage in NTN. (A-D) Groups of 6 to 8 CD11c-DTR/GFP mice (squares) or non-transgenic controls (triangles) that had received 2.5 mg nephrotoxic sheep Ig/g body weight were injected with DT as shown in the protocol in Figure 4D. On day 10, mice were placed in metabolic cages and 24 h urine was collected for determining urinary protein excretion per creatinine (D and F). On the following day, kidneys were taken for histologic analysis of the acute tubulo-interstitial damage (A) and the focal segmental glomerulosclerosis scores (B) and serum samples for calculating creatinine clearance (C). These results are representative for 4 of 5 experiments in groups of 3 to 4 mice. In the fifth experiment, differences were not statistically significant. (E and F) In 2 separate experiments depicted by open or closed symbols, mice received only 2.0 mg nephrotoxic sheep Ig/g body weight, and histologic and urine analysis (F) was performed on day 14. n.s, not significant (P > 0.05; *P < 0.05; **P < 0.01).

somewhat diminished, albeit not significantly (Figure 6A). In addition, the cytokine production of splenic leukocytes in response to sheep Ig was similar (Figure 6B). Thus, our depletion protocol left the magnitude and the polarization of the splenic T cell response unchanged, implying that the systemic immune response against sheep Ig, and thus the autologous phase of NTN, had not been compromised, as intended.

Depletion of DCs in the Immune Effector Phase Aggravates NTN

We next examined the effect of kidney DC depletion on NTN by histology. In the nondepleted nephritic control group, glomeruli focally demonstrated segmental necrosis and extracapillary proliferation (Figure 5, E and G). The tubulo-interstitium showed focal tubular damage, as evidenced by tubuli with flattened epithelium and periodic acid-Schiff (PAS)-positive material in the tubular lumen (Figure 5, E and G). Some tubuli lacked luminal space and showed pronouncedly vacuolated cytoplasm of the epithelium. A sparse monocellular cell infiltrate could be seen in the interstitium (Figure 5, E and G). DC depletion in DT-injected CD11c-DTR/GFP mice led to aggravated tubular damage as compared with DT-injected non-transgenic controls (Figures 5, F and H and 7A). In 2 of 5 experiments, increased extracapillary proliferation/necrosis was noted, whereas no significant differences were seen in the other three experiments (data not shown). No significant changes were seen in the scores for glomerular sclerosis and chronic tubulo-interstitial damage on day 11 (data not shown).

Consistent with the more severe acute tubulo-interstitial damage, creatinine clearance of DC-depleted mice was significantly reduced, indicating compromised kidney function (Figure 7). In addition, urinary protein concentration per creatinine was significantly higher in the depleted group (Figure 7B). These findings indicated that kidney DCs attenuated NTN.

The analysis above was performed on day 11, the day after the second depletion, because DC-depleted mice suffered acute kidney failure in these experiments and had to be killed. To exclude that the depletion maneuver as such caused changes similar to acute tubulo-interstitial damage, we induced NTN with a 20% lower serum dose, which allowed analyzing the mice 4 d after the second depletion, on day 14. In 2 of 2 experiments, aggravated acute tubulo-interstitial damage was seen (Figure 7E), whereas scores for extracapillary proliferation/necrosis, glomerular sclerosis, and chronic tubulo-interstitial damage again showed no significant changes (data not shown). In one of the two experiments, proteinuria per creatinine was determined, showing in 3 of 4 DC-depleted mice higher values than in the 3 nondepleted control (Figure 7F), albeit not to a statistically significant extent.

Immunological Mechanisms With Protective Potential in NTN

To elucidate the mechanisms underlying DC-mediated protection against NTN, we enumerated nTreg, which have previously been reported to attenuate NTN by effects mediated in
the secondary lymphatics.23 Indeed, CD25+ FoxP3+ cells in the largest lymphatic organ, the spleen, were more than 50% less frequent, albeit not statistically significant (Figure 8A). In the kidney, we found very few CD25+ FoxP3+ cells (Figure 8B), consistent with the previous study.23 These numbers were unchanged after DC depletion (Figure 8B), arguing against an effect of DC depletion on intrarenal nTreg.

Finally, we speculated on mechanisms by which DCs might have induced IL-10 in CD4+ T cells. Previous work showed that DCs could do so by producing IL-10 themselves16 or by expressing inducible costimulatory molecule ligand (ICOS-L).39,40 Our finding that CD4+ T cells cocultured with kidney DCs from nephritic mice did not secrete IL-10 unless stimulated with antigen (Figure 2B) implied that the DCs did not secrete this cytokine themselves, at least not in measurable amounts. In contrast, kidney DCs expressed about threefold more ICOS-L on their surface than splenic DCs (mean fluorescence intensities: spleen NTN, 1387; spleen without NTN, 1308; kidney NTN, 4451; kidney without NTN, 3675). The 20% increase of expression on kidney, but not splenic, DCs of nephritic mice (Figure 8, C and D) was consistent with the possibility that IL-10 induction may have occurred via ICOS-L.

**DISCUSSION**

The present study is the first to determine the functional role of kidney DCs in experimental glomerulonephritis. *Ex vivo*, DCs from nephritic kidneys stimulated proliferation of cocultured specific CD4+ T cells and production of the cytokines IFNγ and IL-10. The latter has been shown by many previous studies to be protective in various immune-mediated kidney diseases.24–26,30–32 Importantly, endogenous IL-10 was produced in NTN by unidentified cells and its genetic ablation aggravated disease,33 similar to DC depletion. Our findings demonstrate that renal CD4+ T cells represent one source of endogenous IL-10 and that ICOS-L-expressing kidney DCs stimulated its production, raising the possibility of an attenuating role of DCs in NTN.

To address the functional relevance of DCs, we studied the course of NTN in their absence using CD11c-DTR/GFP mice, which have been widely used to investigate the requirement of DCs in models of infection and immune-mediated disease.34 We chose an experimental protocol that spared DCs in the induction phase of the immune response against sheep Ig and eliminated them only during the immune effector phase. A shortcoming of CD11c-DTR/GFP mice is the inability to deplete DCs over longer time periods because repetitive DC depletions in less than weekly intervals are lethal.35 This problem was less serious than anticipated because we noted that kidney DCs were repopulated more slowly than those in lymphatic tissues,34 consistent with the reported half-life of DCs in healthy kidneys of more than 2 wk.41 Although DC turnover certainly will be faster in inflammation, we found that our depletion protocol cleared the kidneys of DCs effectively and long enough to permit studying the course of NTN in their absence. Importantly, depletion was specific for DCs, as numbers of renal macrophages were not diminished. This control was important because it has recently been shown that certain splenic macrophage subpopulations were depleted in CD11c-DTR mice.35 Under these conditions, ablation of DCs resulted in aggravation of NTN, as documented by increased acute tubulo-interstitial damage and by functional parameters. It is possible that the DCs that induced IL-10 production were especially sensitive to DT and may have been preferentially depleted. A Th1 shift was excluded as an underlying reason because the systemic Ig titer, in particular the IgG2a titers, and the Th1 cytokine production by splenocytes in response to this antigen were not increased.

DC depletion did not aggravate glomerular damage, consistent with the restriction of DCs to the tubulo-interstitium both in
healthy kidneys and in NTN. Thus, DCs might preserve kidney function by attenuating spreading of glomerular injury to the tubulointerstitial compartment, a process known to correlate with the residual kidney function.

DC depletion resulted in an oppositional effect on the course of NTN as compared with macrophage ablation using similar transgenic mice. Thus, renal DCs appeared to act antagonistic to renal macrophages in experimental glomerulonephritis. Both classes of antigen-presenting cells usually express MHC II and thus would be able to interact with, and perhaps to compete for interaction with infiltrating CD4+ T cells. In this way, DCs might prevent CD4+ T cells from stimulating macrophages to perform DTH responses that aggravate NTN. This could also affect interactions of CD4+ T cells with intrinsic renal MHC II+ cells, which has previously been shown to be important in NTN. That study did not exclude that MHC II on other cells were relevant in NTN, and the requirement of macrophages indeed demonstrated the existence of an additional such subset. The present study identifies DC as a further relevant MHC II+ subset, albeit with protective rather than pathogenic effect.

A further explanation for this protection is the induction of Treg. In support of this notion, we noted that DC depletion resulted in a tendency toward reduced nTregs in the spleens of nephritic mice. Since Wolf et al. have recently demonstrated that this particular Treg subtype attenuated NTN by effects mediated in secondary lymphatics, reduced numbers of splenic nTregs theoretically might aggravate disease, for example, by permitting unimpeded generation of pathogenic Th1 effector cells in this site. In contrast, nTregs were scarce in the kidney and unchanged after DC depletion, arguing against their ability to protect the kidney locally. Instead, we found evidence for local protection by a different Treg type, namely, Tr1 cells. Their suppressive function has been studied extensively in allergic asthma and immune-mediated colitis models, where they were induced by local DCs. A role of Tr1 cells in nephritis has not yet been reported. However, the concurrent induction of IFNγ observed here is not a standard feature of Tr1 cells. Instead, this would be consistent with a very recently described CD4+ T cell type that secreted both cytokines during anti-infectious Th1 immune responses. Such IL-10-producing Th1 cells not only cleared for pathogens but terminated the anti-infectious immune response, to prevent collateral organ damage by uncontrolled effector functions. It is conceivable that kidney DCs attempted to use this natural mechanism in NTN, to terminate a harmful Th1 response. This interpretation might help explaining the conflicting results reported on the role of IFNγ in NTN, anti-GBM, and lupus nephritis, where both protective and aggravating effects were observed. Possibly, the role of IFNγ can be modified by the presence of other cytokines such as IL-10, as recently suggested.

Although plausible, our findings do not formally prove that the protective effect of kidney DCs on NTN was mediated by IL-10 induction. Establishing this causal link would require a system, in which DCs cannot induce IL-10 in CD4+ T cells. In asthma models, DCs did so by producing IL-10 themselves or by ICOS-L. The present study provides evidence for a role of the latter molecular mechanism in NTN, which is consistent with previous work showing that ICOS-L blockade aggravated NTN and reduced IL-10 production in splenocytes from nephritic mice. Future studies may determine whether kidney DCs used ICOS-L to attenuate NTN and to what extent this was mediated via IL-10.

In conclusion, we have demonstrated a protective role of kidney DCs in NTN, which may be linked to their ability to express ICOS-L and/or to induce IL-10 production. In addition, DCs in lymphatic organs may contribute to protection by inducing nTregs. These findings identify mechanisms that may be therapeutically exploited to treat immune-mediated kidney disease.

**CONCISE METHODS**

**Mice, Reagents, Nephrotoxic Nephritis Model**

Female 6- to 8-wk-old C57/BL6 and CD11c-DTR/GFP mice were bred and kept in specific-pathogen-free condition at the animal facility of University of Bonn. NSS was generated as described by immunizing sheep with homogenized murine renal cortex in CFA, followed by monthly boosting doses in IFA. The amount of NSS per g body weight used in this study (2.0 or 2.5 mg, kg per g body weight) was within the linear range of a dose response. All animal studies have been approved by Institutional and Government Review Boards. All reagents were from Sigma-Aldrich (Steinheim, Germany), if not specified otherwise.

**Histology**

Light microscopy was performed on 3-μm paraffin sections of PFA-fixed tissue stained by periodic acid-Schiff, and Goldner Trichrome. Kidney damage was histologically scored as described previously. In brief, extracapillary proliferation/necrosis and focal segmental glomerulosclerosis were defined as follows: 0, none; 0.5, <25%; 1, 26% to 50%; 2, 51% to 75%; 3, >75% of a glomerulus showing extracapillary proliferation/necrosis and sclerosis. Injury was evaluated in at least 50 glomeruli per sections. Acute tubulointerstitial damage was defined as follows: 0, no tubular damage, no interstitial edema; 0.5, thinning of the brush border; 1, thinning of the tubular epithelia; 2, denudation of the tubular basement membrane; 3, tubular necrosis. Chronic tubulointerstitial damage was defined as broadening of the basement membrane of tubuli with flattened epithelium, and interstitial matrix increase and/or fibrosis, and was evaluated as follows: 0.5, focal chronic damage; 1, diffuse chronic damage. Tubulointerstitial damage was judged in whole kidney sections including cortex and outer stripe of outer medulla. Tubulointerstitial damage scores were calculated as described.

Immunohistology for MHC II+ cells was performed by staining acetone-fixed cryosections of kidney tissue with biotinylated anti-murine IA and DAB-peroxidase and 3,3′-diaminobenzidine, followed by methyl green counterstaining.

**Isolation of Murine DCs and CD4+ T Cells**

Kidneys and spleens were digested with collagenase (Roche Diagnostics, Mannheim, Germany) and DNAase-I as described. Tubular fragments from digested kidneys were removed by sedimentation.
CD11c⁺ DCs and CD4⁺ T cells were then enriched using nanobead-labeled specific monoclonal antibodies (clones N418 and GK1.5, respectively) (Miltenyi, Bergisch-Gladbach, Germany). Magnetic bead separation was done according to the manufacturer’s instructions. Purity was usually 90% to 95% for DCs and >95% for CD4⁺ T cells. OT-II cells were isolated from spleen and lymph nodes of OT-II mice using nanobead-based kits. Purity was usually about 85%.

**Flow Cytometry**

After treatment with FcBlock (BD-Pharmingen), cells were stained for 15 min on ice with fluorochrome-labeled monoclonal antibodies against CD4 (clone GK1.5), CD8a (53 to 6.7), B220 (RA3-6B2), NK1.1, CD11b (M1/70), CD11c (HL3), I-A⁺ (AF6–120.1) (BD-Pharmingen), CD25 (PG61), FoxP3 (FJK-16s), and ICOS-L (IIK5.3) (Ebiosciences, Karlsruhe, Germany). Dead cells were excluded with Hoechst 33342 or 7-AAD and analyzed with a FACSCalibur (Becton Dickinson, CA).

**In Vitro Cytokine Production**

For determining cytokine induction by DC, 5 × 10⁴ DCs were cocultured with 1 mg/ml OVA in 100 μl DMEM (10% FCS) in 96-well plates for 2 h, then washed and coincubated with 1 × 10⁵ OT-II cells for 3 d. For determining cytokine production by renal T cells, 1 × 10⁵ renal CD4⁺ T cells were cultured for 42 h in 96-well plates coated with 10 μg/ml anti CD3 (clone 2c-11). For determining cytokine production in response to sheep-Ig, 2 × 10⁵ spleen cells were cultured for 72 h with 10 μg/ml sheep globulin. Cytokines in the supernatants were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Duosets, Wiesbaden-Nordenstadt, Germany).

**Miscellaneous Assays**

Serum levels of murine Ig specific for sheep Ig levels were determined by ELISA, proteinuria by Bradford assay (BioRad, Munich, Germany), and creatinine as described. For creatinine clearance, urine was collected over 16 to 24 h to obtain daily creatinine excretion.

**Statistics**

Results are expressed as mean ± SD. Comparisons were drawn using a two-tailed t test (Prism 4, Graphpad Software, San Diego, CA) except for histologic analysis, where the two-sided nonparametric Mann-Whitney U test was used.

**ACKNOWLEDGMENTS**

The authors thank Steffen Jung for CD11c-DTR/GFP mice, Shuo Li for sharing experience with NTN, Felix Heymann for generating NSS, and Juliane Maurer for excellent technical assistance. The authors acknowledge support by the IMMEI flow cytometry core facility and by the House for Experimental Therapy. This work was supported by an institutional BONFOR grant (J.S.) and by the Klinische Forschergruppe 115 and the SFB542 of the Deutsche Forschungsgemeinschaft.

**DISCLOSURES**

None.

**REFERENCES**


