Identification and Functional Characterization of Dendritic Cells in the Healthy Murine Kidney and in Experimental Glomerulonephritis

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Abstract. The kidney tubulointerstitium contains numerous bone marrow-derived antigen-presenting cells, which are often referred to as resident tissue macrophages, although several previous studies had demonstrated characteristics of dendritic cells (DC). In this study, we describe a subset of tubulointerstitial cells expressing the DC marker CD11c. A protocol was established to isolate these cells for *in vitro* analysis. Renal $CD11c^+$ cells resembled splenic DC, but not peritoneal macrophages, in morphology, lysosomal content, phagocytic activity, microbicidal effector functions, expression of T cell costimulatory molecules, and ability to activate T cells. Nevertheless, many $CD11c^+$ renal cells expressed low or intermediate levels of F4/80 and CD11b, indicating that both markers are not absolutely specific for macrophages in the kidney. Subpopulations of renal DC could be distinguished based on their expression of MHC class II and costimulatory molecules and may represent different maturation stages. In nephrotoxic glomerulonephritis, increased numbers of $CD11c^+$ cells showing DC functionality were found in the tubulointerstitium. Focal accumulation was seen within tubulointerstitial mononuclear infiltrates and adjacent to, but not within, inflamed glomeruli. These results are the first to identify and characterize renal $CD11c^+$ cells as DC and to demonstrate marked changes in experimental glomerulonephritis.

Interest in the tubulointerstitial compartment is growing as a result of its pivotal role in the progression of renal disease (1). The cells residing in this compartment are mostly fibroblasts and bone marrow-derived cells of the immune system (2,3). The tubulointerstitial immunocytes have been shown to express MHC class II molecules, indicating that they are professional antigen-presenting cells (APC), *i.e.*, macrophages or dendritic cells (DC). Twenty years ago, before the importance of the extensive system of DC was fully appreciated, the tubulointerstitial immunocytes were found to express the F4/80 molecule, then assumed to be a macrophage marker (4), resulting in their classification as tissue macrophages. Although F4/80 has recently been molecularly characterized (5), its function remains unresolved. It has been shown to be expressed also by some DC subsets (6), in particular those representing earlier differentiation stages (7). Most murine DC populations specifically express the integrin chain CD11c (8). Previous studies have failed to detect $CD11c^+$ cells in the kidney (9,10). Macrophages lack CD11c but possess CD11b, also known as Mac-1. Although CD11b is often used to identify murine macrophages by immunohistochemistry, it is known to be expressed also by granulocytes and DC subsets (8). These and many other DC subtypes have been defined by their expression of various combinations of cell surface markers, but it is unclear whether they represent individual cell types with distinct lineages or differentiation stages of the same cell. Thus, the parameters that unequivocally identify DC remain their morphology and their functionality.

A careful morphologic analysis demonstrated by electron microscopy that tubulointerstitial cells possess features of DC, whereas cells with macrophage morphology were confined to the capsule, the intravasal lumina, and the pelvic wall of healthy kidneys (2). The functionality of these DC-like tubulointerstitial cells was not addressed. The principal DC function is the induction of primary immune responses (11). Their precursors are produced in the bone marrow and then released into the blood stream (12,13), from which they enter tissues. Immature DC, the Langerhans cell in the skin being the best-studied example, capture antigen in peripheral tissues and

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sense "danger" signals (pathogens, tissue damage, local inflammation), which trigger their maturation. Maturation downregulates the phagocytic activity of DC but upregulates the molecular machinery for T cell activation (*e.g.*, MHC and costimulatory molecules) and induces their migration to draining lymph nodes (12). There, DC present antigens to specific T cells. These DC functions, however, can principally be performed by all classes of professional APC, albeit at lesser efficacy. For example, macrophages are most effective at phagocytosis, but far less effective at T cell activation, than DC. Typical macrophage effector functions, such as oxidative burst or nitric oxide (NO) production, are normally not performed by DC. In the present study, we demonstrate a hitherto undescribed kidney cell population expressing DC and macrophage markers and characterize them in the healthy and inflamed kidney as DC.

Materials and Methods

Mice, Reagents, and the Nephrotoxic Nephritis Model

Female C57/BL6 and Balb/c mice 6 to 8 wk old in specific pathogen-free conditions were purchased from Bomholtgard (Denmark). All experiments were in accordance with local animal ethics procedures. Reagents were from Sigma-Aldrich (Steinheim, Germany) if not specified otherwise. Nephrotoxic nephritis (NTN) was induced by injection of 6 μl of nephrotoxic sheep serum per gram of mouse body weight as described (14,15).

Immunohistology

For immunohistochemistry, tissues were fixed for 3 d in Immunohistofix (Intertiles, Bruxelles, Belgium) and then embedded in Immunohistowax (Intertiles) at 37°C according to the manufacturer's instructions (16). Dewaxed sections were incubated with 3% H_2O_2, BR blocking reagent (Roche, Mannheim, Germany), and Fc-Block® (Pharmingen, Heidelberg, Germany). Primary monoclonal antibodies were biotinylated anti-CD11c (clone HL-3), anti-Ia^b (AF6-120.1), anti-CD11b (Mac-1) (all from Pharmingen), and anti-F4/80 (CI:A3-1; Caltag, CA). As isotype controls, biotinylated antibodies against irrelevant antigens were used (Pharmingen). Bound antibody was revealed with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and 3,3'-diaminobenzidine (DAB). Slides were counterstained with methyl green.

Preparation of Splenic DC, Renal $CD11c^+$ Cells, and Peritoneal Macrophages

Previously described methods for APC isolation from spleens (17,18) were modified to isolate $CD11c^+$ cells from murine kidneys. Kidneys were finely minced and digested for 45 min at 37°C with 2 mg/ml collagenase D (Roche) and 100 μg/ml DNAse I in RPMI 1640 medium (Life Technologies, Karlsruhe, Germany) in 10% heat-inactivated FCS (PAA Laboratories, Linz, Austria) containing 10 mmol/L Hepes. Cell suspensions were filtered through 30-μm nylon mesh, washed with HBSS without Ca^{2+} and Mg^{2+} (Life Technologies) containing 10 mmol/L EDTA, 0.1% BSA, and 10 mM Hepes, and adjusted with NaCl to mouse tonicity. A density centrifugation was performed at 1700*g* for 20 min at 4°C using 1.080 g/ml Nycodenz (Axis Shield, Oslo, Norway). The interphase cells were harvested, Fc receptors were blocked with Fc-Block® (Pharmingen), and $CD11c^+$ cells were isolated with microbead-labeled specific monoclonal antibodies (clone N418) (Miltenyi, Bergisch-Gladbach, Germany). Magnetic bead separation was done according to the manufacturer's instructions.

Splenic DC were isolated using previously described methods (17,18) and enriched with microbead-labeled anti-CD11c (Miltenyi). Peritoneal macrophages were obtained by flushing the peritoneal cavity with HBSS without Ca^{2+} and Mg^{2+} containing 10 mM EDTA and 0.1% BSA. For most experiments, macrophages were further enriched with microbead-labeled anti-CD11b (Miltenyi).

Flow Cytometry

After treatment with Fc-Block® (Pharmingen), isolated cells were stained for 15 min on ice with fluorochrome-labeled monoclonal antibodies specific for CD11c, I-A^b, CD11b, CD80 (16-10A1), CD86 (PO3) (all from Pharmingen), and F4/80 (Caltag). For staining of lysosomes, cells were cultured for 15 min with Lysotracker® green (Molecular Probes, Leiden, Netherlands) in culture medium. Dead cells were always excluded with propidium iodide (PI). Multicolor flow cytometry was performed on a Becton Dickinson FACScalibur®.

Phagocytic Activity

Isolated cells (1×10^5) were cocultured with 1 mg/ml FITC-labeled dextran (40,000 kD; Molecular Probes) for 45 min. As a control for nonspecific dextran attachment, 0.02% azide was added or cells were cultured at 4°C to stop energy-dependent cellular functions. To determine phagocytic activity, the uptake of fluorescence beads was detected by multicolor flow cytometry.

NO Release

NO production was measured as described (19). Briefly, 1×10^5 cells were incubated in 200 μl of culture medium without phenol red containing 10 ng/ml IFNγ and varying LPS concentrations. After 48 h, 50 μl of 14 mmol/L 4'4-diaminodiphenylsulfone and 50 μl of 4 mmol/L *N*-ethylenediamine were added to the culture supernatant, and NO was detected by measuring the OD at 560 nm on a Dynatech MR5000 reader (Dynatech, Chantilly, VA).

Alloresponse

Single cell suspensions of lymph nodes and spleens of Balb/c mice were enriched for T cells by nylon wool adhesion or negative selection using a T cell isolation kit (Miltenyi). T cell purity was typically >95%. T cells (10×10^6) were incubated for 8 min at 37°C with 5 μmol/L 5,6-carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes) in RPMI 1640 culture medium (Life Technologies) containing 10% heat-inactivated FCS, 50 μmol/L 2-mercaptoethanol, 100 μg/ml streptomycin, and 100 U/ml penicillin. CFSE-labeled T cells (4×10^5) were cocultured with 1×10^5 APC (DC or macrophages) from C57/BL6 mice. After 72 h, cells were stained for CD4 (L3T4) or CD8 (Ly-2; Pharmingen), and CFSE dilution was analyzed by flow cytometry.

Results

The Kidney Contains Multiple $CD11c^+$ Cells That Are Distinct from $CD11b^+$ and $F4/80^+$ Cells

CD11c is widely used as a marker for murine DC in lymphatic tissues. Previous studies have failed to detect $CD11c^+$ cells in the kidney (9,10,16). Using commercially available antibodies, we also did not detect such cells in paraffin sections of the kidney and spleen. In contrast, a recently developed immunohistological technique using a zinc-based formalin-

free fixative in combination with an embedding wax that melts at lower temperature and thus avoids heating of the tissue to 56°C (16) revealed multiple CD11c$^+$ cells in the tubulointerstitium of the renal cortex (Figure 1B). CD11c$^+$ cells were located in the tubulointerstitial spaces and close to, but mostly outside of, peritubular capillaries. They possessed dendritic processes that protruded into the peritubular spaces. Only occasionally were CD11c$^+$ cells seen in the glomeruli, and these were located within capillary loops. To ensure that this technique specifically detected CD11c$^+$ cells, splenic sections were stained using the same protocol. Here, CD11c$^+$ cells were detected in their typical locations, the T cell areas and the marginal zone of the white pulp (Figure 1A).

To compare CD11c$^+$ cells with macrophages, we stained for the markers F4/80 and CD11b using the same technique. In spleen sections, large, round, mononuclear cells highly positive for CD11b (CD11b^{hi}) were located in the marginal zones and the red pulp, the typical locations of macrophages (Figure 1C, thick arrow). In addition, cells weakly positive for CD11b (CD11b^{lo}) with dendritic morphology were found in the T cell areas (Figure 1C, thin arrow), where the CD11c$^+$ cells were found (Figure 1A), compatible with splenic CD11b$^+$ DC (8). F4/80$^+$ cells were prominent in the splenic red pulp but not in T cell areas (Figure 1E). In kidney sections, the glomerular capillaries contained large, round, mononuclear CD11b^{hi} cells (Figure 1D, thick arrow) in a frequency of approximately one cell per 5 to 10 glomeruli. Occasionally, such cells were seen in tubulointerstitial capillaries. CD11b^{lo} cells with dendritic morphology were found in the extravascular tubulointerstitium (Figure 1D, thin arrow) at a frequency below that of CD11c$^+$ cells (Table 1). The entire tubulointerstitium showed prominent staining for F4/80 (Figure 1F), as described previously (4). The number of renal F4/80$^+$ cells was approximately three times higher than that of CD11c$^+$ cells, but cell numbers in individual animals varied considerably, resulting in high SD (Table 1).

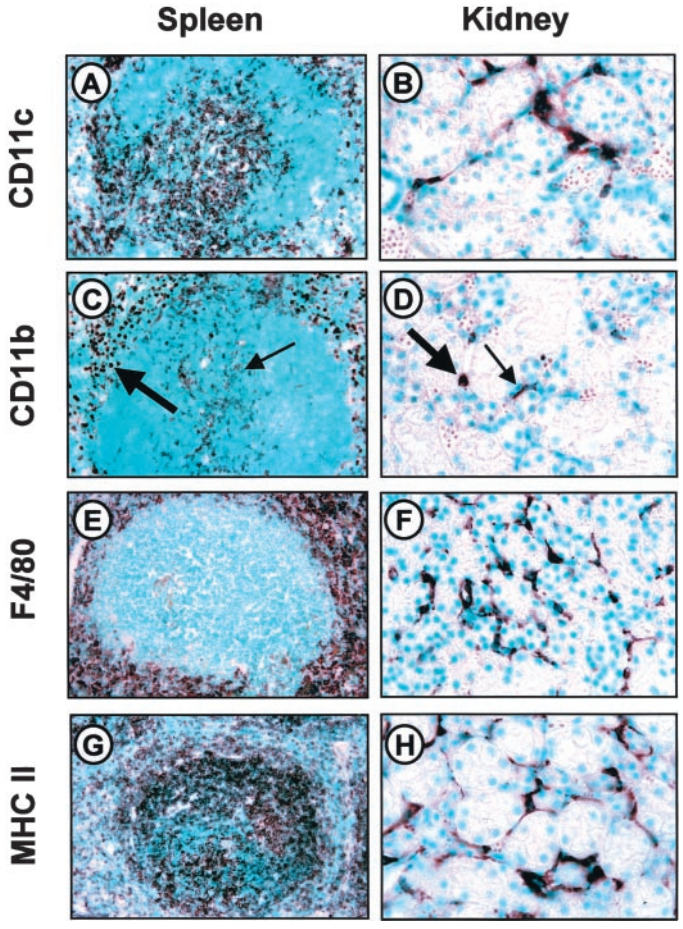

Figure 1. Localization of CD11c$^+$, F4/80$^+$, CD11b$^+$, and MHC II$^+$ in the kidney. Immunohistowax sections of the spleen (A, C, E, and G) and kidney (B, D, F, and H) were stained for CD11c (A and B), CD11b (C and D), F4/80 (E and F), and MHC class II (G and H). The thin arrows in C and D indicate CD11b^{lo} cells in the splenic T cell area and the renal tubulointerstitium, respectively. The thick arrows indicate large, round CD11b^{hi} cells in the marginal zone of the splenic white pulp and in a renal tubulointerstitial vessel. Original magnification: ×100 for the spleen; ×400 for the kidney.

Professional APC generally express MHC class II (8,11). Immunohistowax sections of the spleen revealed large mononuclear MHC II$^+$ macrophages in the red pulp and numerous smaller MHC II$^+$ cells in the white pulp (Figure 1G), representing DC in the T cell areas or B cells in lymphatic follicles. In the kidney, MHC II$^+$ cells were detected in numbers (Table 1) and locations similar to those of F4/80$^+$ cells (Figure 1H), compatible with previous studies (4,20,21).

Isolation and Phenotypical Characterization of Renal CD11c$^+$ Cells

To quantitatively assess expression levels and the coexpression of cell surface markers on CD11c$^+$ cells, we developed a technique to isolate these cells from the kidney and to analyze them by multicolor flow cytometry. This isolation technique was based on existing protocols for APC isolation from the spleen involving collagenase digestion and density centrifugation. Splenic CD11c$^+$ DC usually possess a density of less than 1.080 g/ml, whereas the isolation of macrophages requires a higher density (18). Renal CD11c$^+$ cells were present exclusively in the low-density fraction (data not shown), but purity was usually less than 10% (data not shown). Overnight adherence on plastic to increase the purity of APC was avoided here, because it resulted in the death of many renal CD11c$^+$ cells and caused phenotypical changes in the surviving cells (data

Table 1. Cell numbers in kidney sections[a]

Marker	Cell No.
CD11c	35 ± 13
CD11b	13 ± 6
F4/80	102 ± 33
MHC II	109 ± 28

[a] Immunohistowax sections of eight healthy B6 mice were stained for CD11c, CD11b, F4/80, and MHC class II. Numbers of positive cells per low-power field (original magnification × 100) were counted.

not shown). Instead, we added an additional isolation step using CD11c-specific microbeads. This technique yielded 0.05 to 0.1 $\times$ 10^6 viable $CD11c^+$ cells per kidney, compared with 1 to 3 $\times$ 10^6 $CD11c^+$ cells per spleen. Peritoneal macrophages isolated from the peritoneal cavity were also used as a control cell population. Per peritoneal cavity, 2 to 5 $\times$ 10^6 $CD11b^+$ cells were obtained. Importantly, no inflammatory stimuli were instilled.

These three cell populations were examined by flow cytometry. The optical scatter properties for renal $CD11c^+$ cells were comparable to those of splenic DC, whereas macrophages displayed larger side scatter (Figure 2A), indicating higher laser light diffraction at intracellular organelles such as lysosomes. To assess the purity of our isolation method, the proportion of $CD11c^+$ cells among the viable cells was determined by immunofluorescence using a monoclonal antibody for a different CD11c epitope and compared with an appropriate isotype control. Typically, approximately 95% of renal

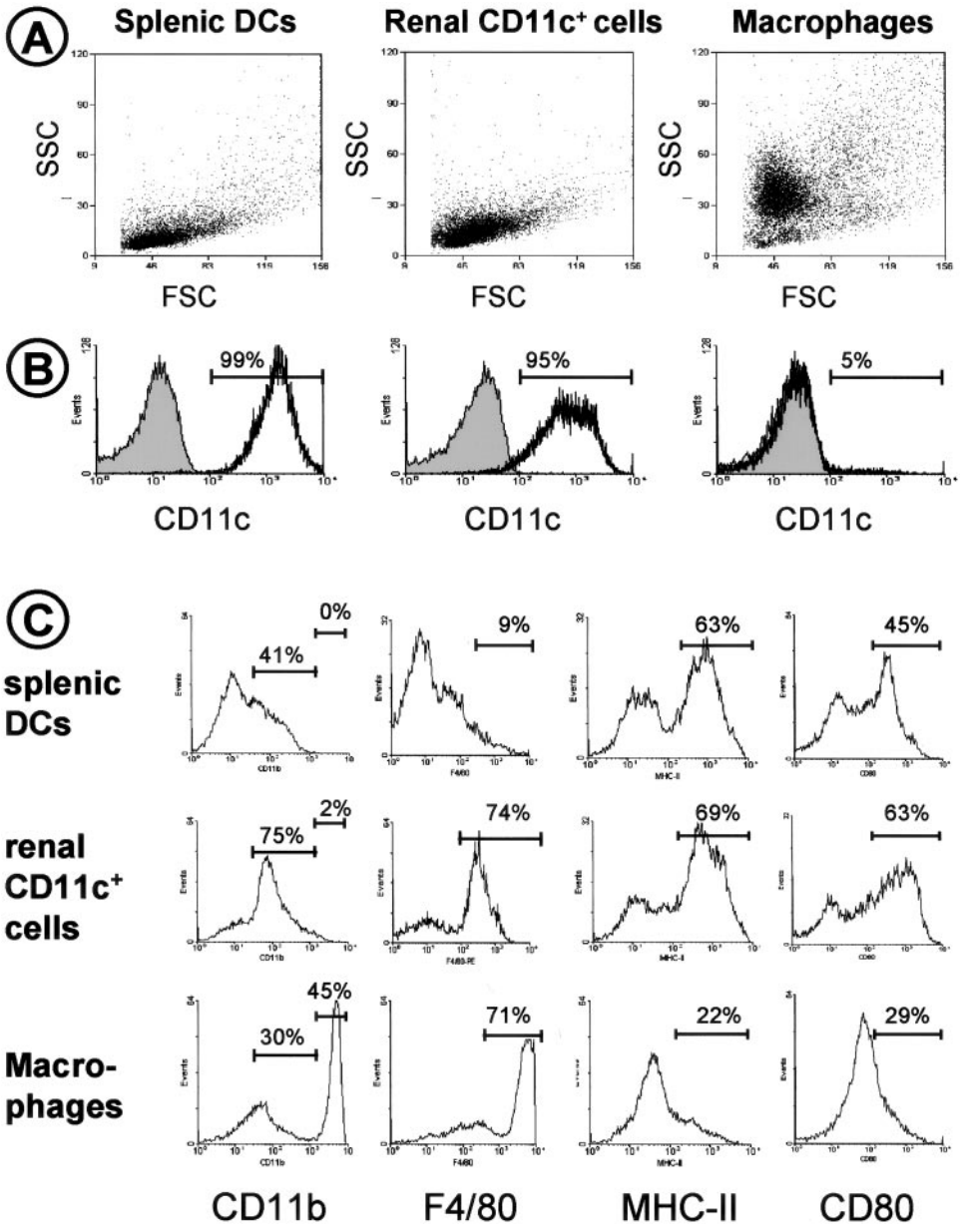

Figure 2. Flow cytometry comparison of renal $CD11c^+$ cells with splenic dendritic cells (DCs) and peritoneal macrophages. (A) Optical forward (FSC) and side (SSC) scatter dot plots. (B) Purity of isolated cells was assessed by staining with biotinylated anti-CD11c (clone HL-3) (thick line) or isotype control (thin line with gray shading) after blocking of Fc receptors. The numbers indicate the proportion of cells expressing more CD11c than the isotype control. (C) Expression of CD11b, F4/80, MHC class II, and CD80 was determined on renal $CD11c^+$ PI^- cells, splenic DCs, and peritoneal macrophages after blocking of Fc receptors. The numbers in the histograms give the proportion of cells expressing each marker. For CD11b, the proportions of cells displaying low and high levels are given separately. The results are representative of four individual experiments.

cells and more than 98% of splenic cells were CD11c$^+$, with splenic cells expressing higher levels of this molecule (Figure 2B). Few peritoneal macrophages expressed CD11c, and expression levels were low (Figure 2B).

Next, we compared the expression of the other cell surface markers examined in Figure 1 on CD11c$^+$ cells (Figure 2C). In the spleen, both CD11b$^-$ and CD11b^{lo} DC were visible, as described (8). Renal CD11c$^+$ cells were mostly CD11b^{lo}, compatible with the finding of CD11b^{lo} cells in kidney sections (Figure 1D). The CD11b^{hi} cells detected by histology (Figure 1, C and D) were found only among peritoneal macrophages and neither in splenic nor renal cell preparations, indicating that such cells were excluded by our isolation protocol. Likewise, high levels of F4/80 were expressed only on peritoneal macrophages, whereas only some splenic DC expressed this marker at low levels (Figure 2C), consistent with the absence of F4/80$^+$ cells in splenic DC areas (Figure 1E). Interestingly, most of the renal CD11c$^+$ cells expressed high F4/80 levels, albeit not as high as those of macrophages (Figure 2C). The levels of MHC class II and of the costimulatory molecules CD80 (Figure 2C) and CD86 (data not shown) on renal CD11c$^+$ cells and splenic DC varied over more than 2 orders of magnitude. In contrast, macrophages generally expressed low levels of these molecules. Expression of costimulatory molecules by some renal CD11c$^+$ cells was higher than that of splenic DC. This consistent finding was not artificially induced by our DC isolation method, because similar levels were found when azide was added during isolation (data not shown), which stops all energy-dependent cell functions, such as surface molecule upregulation or downregulation.

Dot-plot analysis of renal CD11c$^+$ cells revealed cellular subpopulations differing in MHC II expression. A distinct population of 4 to 6% of CD11c$^+$ cells was devoid of MHC II expression (Figure 3A). These cells colocalized with a similar CD11c$^+$ MHC II$^-$ population in the blood (Figure 3B), which recently was identified as a common DC precursor population (13). To determine whether the renal CD11c$^+$ MHC II$^-$ cells were blood-borne, mice were perfused thoroughly before the kidneys were taken. This did not affect the number of CD11c$^+$ cells isolated per kidney (data not shown) but reduced the CD11c$^+$ MHC II$^-$ population slightly to 3 to 5% (Figure 3C), indicating that this renal cell population only partially represented blood-borne DC precursors. Renal CD11c$^+$ cells expressed MHC II$^+$, in good correlation with the costimulatory molecules CD80 (Figure 3D) and CD86 (data not shown), suggesting that the populations differing in MHC expression may represent DC maturation stages.

The histogram analysis shown in Figure 2C suggested the existence of subpopulations of renal CD11c$^+$ cells differing in their expression of CD11b and F4/80. Dot-plot analysis verified this hypothesis by revealing distinct populations of F4/80$^-$ and F4/80$^+$ renal CD11c$^+$ cells (Figure 3E), which could be further subdivided into cells expressing CD11b or not (Figure 3F). These findings demonstrate that renal CD11c$^+$ cells were not a homogeneous cell population.

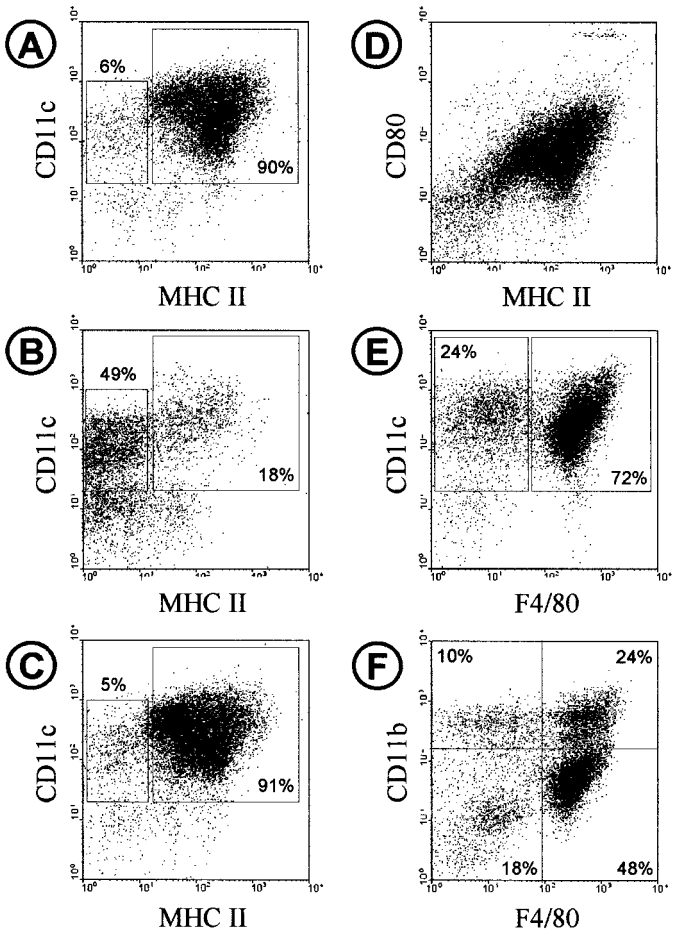

Figure 3. Subpopulations of renal CD11c$^+$ cells. CD11c$^+$ isolated from the kidney (A and C to F) or from blood (B) were analyzed by multicolor flow cytometry. In C, donor mice were perfused with 50 ml of PBS before the kidney was taken. Shown are typical dot-plot analyses of MHC class II *versus* CD11c (A to C), MHC class II *versus* CD80 (D), and F4/80 *versus* CD11c (E) expression on PI$^-$ cells. F shows F4/80 *versus* CD11b dot plot analysis of the cells in E after gating for CD11c$^+$ PI$^-$ events. The numbers indicate the cellular proportions in each region. Shown are results representative of four experiments.

Functional Comparison of Renal CD11c$^+$ Cells with DC and Macrophages

The data described above showed that renal CD11c$^+$ cells expressed both macrophage and DC markers. Because of this conflicting marker status, we decided to base the classification of renal CD11c$^+$ cells on their functionality. We examined typical macrophage functions, such as phagocytosis, lysosomal content, or NO production in response to microbial stimuli, and as a typical DC function, T cell activation. To ensure comparability, all cell populations were prepared using microbeads. For macrophages, CD11b-specific beads were used, because these cells did not express CD11c (Figure 2C) (8)).

Phagocytosis was determined by flow cytometry evaluation of FITC-labeled dextran uptake. Here, only macrophages showed strong activity. Splenic DC and renal CD11c$^+$ cells showed significant FITC-labeled dextran uptake, but this was 1

order of magnitude lower than that of macrophages (Figure 4A). Phagocytosed material is intracellularly degraded in lysosomes. The lysosomal content can be measured with fluorescent dyes that accumulate after uptake into acidic compartments. In this assay, macrophages showed higher lysosomal contents than did splenic DC and renal CD11c$^+$ cells (Figure 4B). T cells, which do not possess lysosomes, served as a negative control. NO production was tested by culturing cells with LPS and IFNγ and by measuring NO in the supernatant. Macrophages produced large amounts of NO, in contrast to splenic DC and renal CD11c$^+$ cells (Figure 4C). These data demonstrate that renal CD11c$^+$ cells did not show macrophage functionality, excluding the possibility that the subpopulations identified above represented macrophages.

The ability to activate T cells was determined in a mixed lymphocyte culture using responder T cells from Balb/c mice. Their proliferation was assessed using the CFSE dilution technique. Division of CFSE-labeled cells results in the dilution of this fluorescent dye. The number of divided cells indicates T cell proliferation (22). Both splenic DC and renal CD11c$^+$ cells activated T cells, with splenic DC consistently being more effective (Figure 5, A and B). In contrast, peritoneal macrophages induced less T cell proliferation (Figure 5F), consistent with their low expression of MHC class II (Figure 2C). No proliferation resulted when congenic C57/BL6 T cells were added to renal DC (Figure 5E) or to the other cell populations (data not shown), indicating that T cell activation was MHC-restricted. These results demonstrate that renal CD11c$^+$ cells possessed DC activity. However, the principal DC function, namely T cell activation, cannot be determined for individual APC. Thus, it is possible that some renal CD11c$^+$ cells did not contribute to the observed T cell activation and may represent CD11c$^+$ non-DC devoid of macrophage functionality.

Localization and Functionality of Renal CD11c$^+$ Cells in Nephrotoxic Glomerulonephritis

To investigate CD11c$^+$ cells in inflammatory kidney disease, we induced NTN by injection of nephrotoxic sheep serum as described (14,15). Seven days after disease induction, the mice had developed severe proteinuria and showed severe glomerular damage with focal glomerular necrosis and tubulointerstitial damage, including tubular atrophy and mononuclear infiltrates (Figure 6, B and C). The number of renal CD11c$^+$ cells was increased in Immunohistowax sections (Figure 6, A and B) and after isolation from the kidney using our protocol (healthy, 61,000 $\pm$ 34,000 per kidney, *versus* NTN, 391,000 $\pm$ 149,000 per kidney; $n = 4$). In addition, marked changes in the localization were seen: severely damaged glomeruli showed periglomerular accumulation of CD11c$^+$ cells (Figure 6C). Interestingly, also in NTN despite significant glomerular injury, intraglomerular CD11c$^+$ cells were almost completely absent (Figure 6C and data not shown). Focal accumulation of CD11c$^+$ cells was seen within mononuclear tubulointerstitial infiltrates (Figure 6B) and within the adventitia of small intrarenal arteries (Figure 6D). These morphologic changes were dose-dependent, because in mice that received a 33% lower dose of nephrotoxic sheep serum, changes

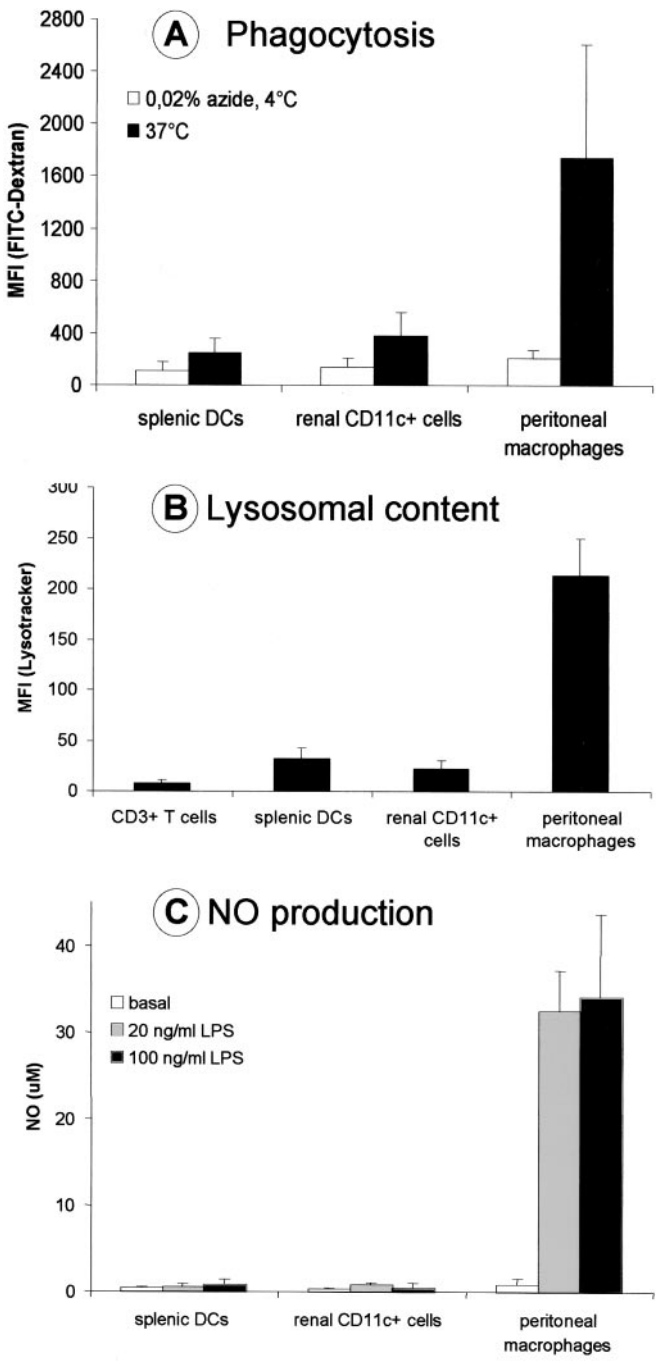

Figure 4. Macrophage functionality by renal CD11c$^+$ cells, DCs, and macrophages. (A) Renal CD11c$^+$ cells, splenic DCs, and peritoneal macrophages were cocultured with FITC-labeled dextran in the absence (black bars) or presence (white bars) of azide. After 45 min, the uptake of fluorescence beads was determined by flow cytometry. (B) T cells (negative control), splenic DCs, renal CD11c$^+$ cells, and peritoneal macrophages were cultured with Lysotracker® green in culture medium at 37°C (Molecular Probes). After 15 min, fluorescence accumulation in lysosomes was detected by flow cytometry. In A and B, the geometric means of the fluorescence intensities (MFI) of 10^4 CD11c$^+$ (for renal and splenic cells) and 10^4 CD11b$^+$ (for macrophages) cells are shown. (C) Renal CD11c$^+$ cells, splenic DCs, and peritoneal macrophages were cultured with IFNγ and 0 (white bars), 20 (gray bars), or 100 (black bars) ng/ml LPS. After 2 d, nitric oxide (NO) was measured in the culture supernatant. The results are representative of three individual experiments.

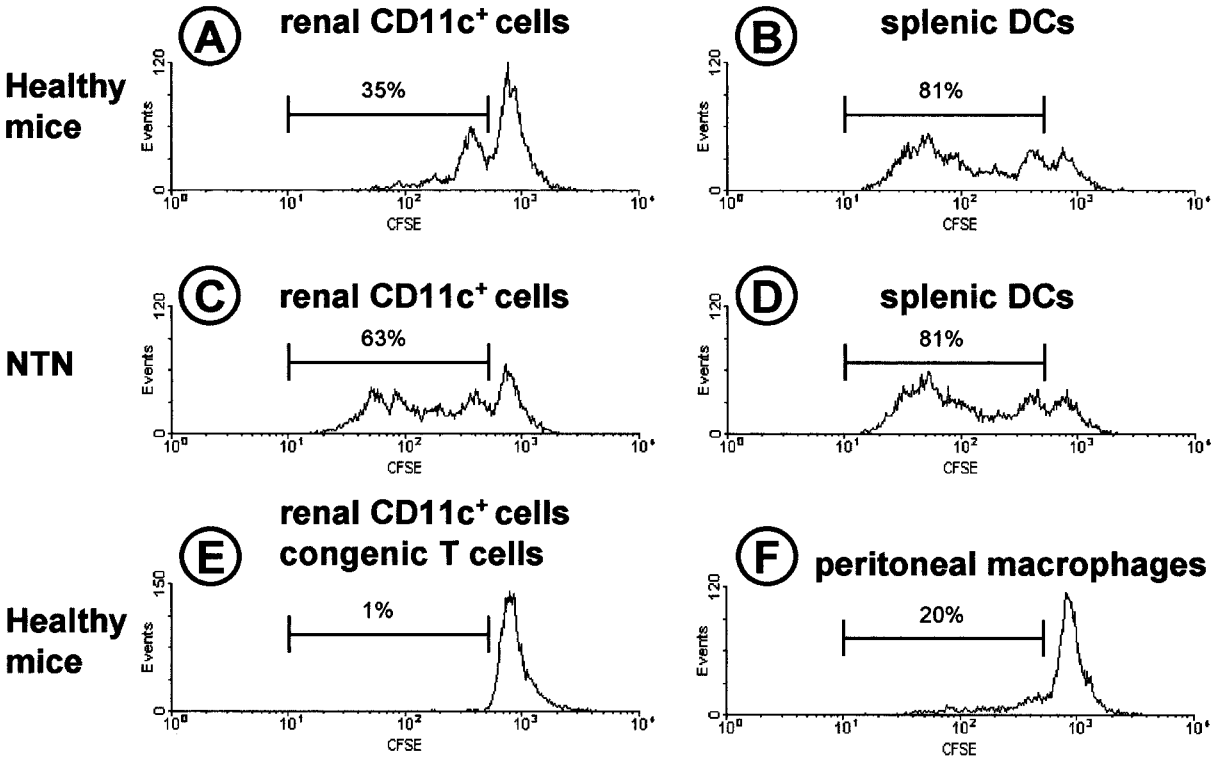

Figure 5. Ability to activate T cells. Renal CD11c$^+$ cells (A, C, and E), splenic DCs (B and D), and peritoneal macrophages (F) from C57/BL6 donor mice were cocultured with 5,6-carboxyfluorescein succinimidyl ester (CFSE)-labeled T cells from Balb/c mice (A to D and F) or C57/BL6 mice (E). After 3 d, T cell proliferation was determined by flow cytometry. Shown are the CFSE levels of the CD3$^+$ cells in culture. The proportion of proliferated T cells showing reduced CFSE intensity is indicated by the numbers. The results are representative of four individual experiments. NTN, nephrotoxic nephritis.

were similar but less severe. Animals that received a 50% higher dose showed anuric kidney failure at day 7 and signs of wasting, and their kidneys contained very few CD11c$^+$ cells (data not shown).

CD11c$^+$ cells isolated from mice with NTN induced T cell proliferation (Figure 5C), demonstrating that these cells still possessed DC functionality. This ability was increased compared with that of renal CD11c$^+$ cells from healthy mice (Figure 5A), suggesting that renal CD11c$^+$ cells were activated. NTN did not increase the T cell proliferation induced by splenic DC (Figure 5, B and D), demonstrating organ specificity. Macrophage functions of renal CD11c$^+$ cells such as phagocytic ability and NO production were not significantly changed in NTN (data not shown).

Discussion

Bone marrow-derived APC were identified in the kidney tubulointerstitium more than 20 yr ago (4). Their intense staining for the macrophage marker F4/80 (4) and failed attempts to detect the DC marker CD11c (9,10) resulted in their classification as resident tissue macrophages. Using an alternative, recently published immunohistochemical method (23), the present study clearly demonstrates expression of the DC marker CD11c on numerous tubulointerstitial cells. These cells were less numerous than F4/80$^+$ cells, and there was considerable overlap, because most of the CD11c$^+$ cells expressed F4/80. Many studies have attempted to classify APC by cell surface markers. In particular, numerous DC subpopulations have been defined by their expression of surface markers, such as myeloid DC expressing CD11b or immature DC subsets positive for F4/80 [6–9,17]. Most of these studies, however, have studied DC in lymphatic tissues, which are easier to isolate. In nonlymphoid tissues, in particular in the kidney, it remains to be shown that CD11c$^+$ cells are DC. There is also functional overlap between macrophages and DC, so that the definition of clear barriers is difficult, and maybe even impossible. For example, principal DC functions such as the activation of T cells can also be performed by macrophages, albeit less efficiently, and depending on the activation and differentiation state. Likewise, some DC populations can perform bactericidal functions normally ascribed to macrophages (24). At least *in vitro*, these cell types could be converted to each other by cytokines (25). Consequently, it has been suggested that the macrophage-DC dichotomy be replaced with a differentiation continuum of mononuclear phagocyte cells, with DC and macrophages as functional extremes (26). Our findings are consistent with this idea. We determined both macrophage and DC functions for renal CD11c$^+$ cells compared with defined populations of DC and macrophages. Splenic DC were effective T cell activators with little phagocytic and bactericidal activity, as expected. In contrast, peritoneal macrophages were very efficient at these latter functions, whereas their ability to

activate T cells was small. Compared with these functional extremes, renal CD11c$^+$ cells showed functionality very close to that of DC, which in our opinion allows the classification of renal CD11c$^+$ as DC. Slightly higher phagocytic activity and less efficient T cell activation were noted and may indicate some macrophage functionality or immature DC functionality. These results do not exclude that renal CD11c$^+$ cells may perform macrophage effector functions under certain situations, such as renal infections (24). In the healthy kidney and in NTN, however, the functionality of renal CD11c$^+$ cells was confined to that of DC, despite the expression of CD11b and F4/80. These findings have implications for the use of these markers in immunohistochemistry. They are not absolutely specific for macrophages in the kidney, because most CD11c$^+$ cells also expressed F4/80. Consequently, a fraction of the tubulointerstitial F4/80 cells must be DC. This is a remarkable difference from lymphoid tissues such as the spleen, in which few DC expressed F4/80 and only at low levels.

Renal CD11c$^+$ cells did not represent a homogeneous cell population. A CD11c$^+$ MHC II$^-$ subset resembled DC precursors. The expression patterns of MHC class II and costimulatory molecules suggested that different DC maturation or activation stages were present in the kidney (8). These results are consistent with a previous study showing that MHC II$^+$ cells from the kidney performed some DC functions (9). CD11c, however, was not detected in this previous study; thus, their equivalence to the renal CD11c$^+$ cells investigated here is unclear, in particular because the kidney was shown to contain non-bone marrow-derived cells expressing MHC II, such as endothelial cells (20,21,27). Additional subpopulations were identifiable when expression of CD11b and/or F4/80 was examined on renal CD11c$^+$ cells. The CD11b$^-$ CD11c$^+$ population is unlikely to be related to CD11b$^-$ CD11c$^+$ "lymphoid" DC in lymphatic tissues (8), because renal CD11c$^+$ cells did not express the CD8α molecule (D. Benke, unpublished observations), which is characteristic for these DC (8).

Also in experimental glomerulonephritis, CD11c$^+$ cells showed DC functionality, which was even higher than that of cells from healthy kidneys. Inflammation is known to induce the recruitment of DC to affected nonlymphoid organs and also their emigration from these organs to draining lymph nodes (8,11). For MHC II$^+$ cells in the kidney, this has been reported after systemic LPS injection (12,28). Similar dynamic changes may occur to renal CD11c$^+$ DC in NTN. The accumulation of these cells in kidney arterial walls observed in the present study may reflect blood-borne DC precursors entering the inflamed kidney. The result was a marked increase in renal DC numbers in NTN. Interestingly, these cells were preferentially attracted to inflammatory sites, suggesting that soluble mediators such as inflammatory chemokines are crucial in DC recruitment (29). The functional role of renal DC in NTN remains to be elucidated. They may be involved in the resolution of inflammation, but they may also aggravate disease, similar to pulmonary DC, which have recently been shown to contribute to the perpetuation of asthma bronchiale (30). Experiments addressing these questions will be possible based on the present study, as soon as techniques to specifically deplete DC *in vivo* become available.

In conclusion, we have described renal CD11c$^+$ cells, characterized their phenotype and functionality as that of DC, and demonstrated striking changes in experimental glomerulonephritis.

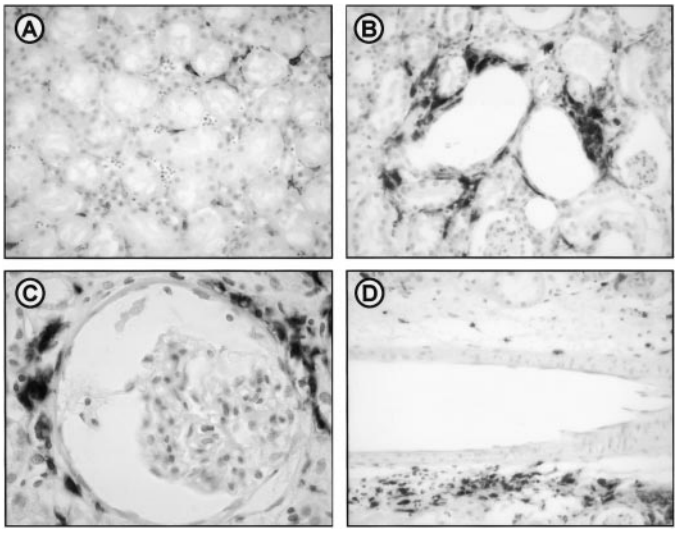

Figure 6. Localization of renal CD11c$^+$ cells in NTN. Seven days after induction of NTN, Immunohistowax sections were stained for CD11c. Shown are low magnifications (×200) of the tubulointerstitium healthy, untreated (A) *versus* diseased animals (B) and higher magnifications (×400) of periglomerular (C) infiltration and of a small intrarenal artery (D). Note the increased numbers of CD11c$^+$ cells at sites of tubulointerstitial (B) and glomerular (C) damage and the perivascular adventitial infiltrate of CD11c$^+$ cells (D). These images illustrate representative changes seen in three experiments in groups of four to five mice.

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