Dendritic Cells in the Kidney

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ABSTRACT

Dendritic cells (DC) in nonlymphoid organs function at the crossroads of innate and adaptive immunity, self-tolerance, and tissue homeostasis. This review provides an overview of the study of DC in the kidney, tracing its history leading to the current knowledge of the origins, migration, and function of renal DC. Together, these studies suggest that renal DC play a critical role in the health and disease of the kidney, opening the way to direct targeting of renal DC for therapeutic benefit.

Dendritic cells (DC) have been extensively studied in lymphoid and many nonlymphoid organs in the past three decades but have received comparatively little attention in the kidney. One reason for this disparity has been an apparent difficulty in identifying, isolating, and manipulating renal DC (rDC) in vitro and in vivo to gain a better understanding of their function in the kidney. These barriers to the study of rDC have fallen in recent years, and it is now clear that rDC exist in intimate communication with the entire renal parenchyma, constantly surveying for and responding to local environmental cues. Here, we review the history of the identification of the rDC network; the origins and migration of rDC at steady state; and the function of rDC in innate and adaptive immunity, self-tolerance, and homeostasis of the renal parenchyma.

IDENTIFICATION OF THE RDC NETWORK

Ground-breaking studies in the 1970s by Steinman and colleagues1,2 identified and functionally segregated “dendritic cells” from other immunocytes in lymphoid organs. This led to the discovery that splenic DC potently stimulated the proliferation of T lymphocytes in mixed leukocyte reactions and expressed surface Ia antigens,3 identifying DC as MHC class II+ rDC in rat kidney were subsequently recognized in 1981 by the existence of resident MHC class II+ cells with stellate and “mononuclear phagocyte” morphology in the renal interstitium4,5 and mesangium.6 These anatomic locations were subsequently confirmed by electron microscopy7,8; however, proof that rDC alloactivate T lymphocytes, similar to DC isolated from lymphoid tissues, was not reported until 19949 or substantiated until more recent-ly10–12 after the ability to isolate rDC via CD11c (the integrin αc chain) expressed by differentiated mouse DC.13 Similar to other DC, differentiated rDC were also characterized as “immature” versus “mature” on the basis of their low versus high expression of co-stimulatory molecules, respectively.

Several functional subsets of DC have now been phenotyped,13–18 and some of these subsets have been identified in the kidney using defined markers (Table 1). Within normal mouse kidneys, >90% of CD11c+ rDC are negative for CD8α and CD45RA (B220),10,12,19 indicating that the majority of mouse rDC are of the myeloid lineage and major constituents of the mononuclear phagocyte system within the kidney.19,20 Small numbers of lymphoid (CD11c+CD8α−B220+; this subset has been identified in mice but not in humans13) and preplasmacytoid (CD11c+CD8α−B220+) rDC have also been detected within mouse kidneys.10,19 Within normal human kidneys, myeloid (BDCA-1+DC-SIGN+ and BDCA-1+DC-SIGN−) and preplasmacytoid (BDCA-2+DC-SIGN+) rDC were recently identified, and on the basis of the quantitative area of positive staining for BDCA-1 versus BDCA-2 in thin sections, preplasmacytoid rDC represent a greater fraction of total rDC in normal human kidneys as compared with normal mouse kidneys (up to 20% versus <10%, respectively).10,19,21 Whether mouse or human kidneys harbor or recruit any of the most recently defined functional DC, such as IFN-producing killer DC22,23 inflammatory TNF-α-inducible nitric oxide synthase–producing DC,24,25 and tolerogenic IDO-expressing DC26–31 is unclear.

Unequivocal evidence that rDC form a true anatomic surveillance network within the renal parenchyma, rather than a random dispersion at steady state, was determined by examination of rDC in situ in the kidneys of CX3CRI+/− mice (Figure 1).18 Under intravital and confo-
cal microscopy from the capsule to the papilla of kidneys in CX3CR1<sup>GFP</sup>/+ mice, stellate-shaped myeloid rDC form a contiguous network throughout the entire interstitium, encasing all nephrons.19 Confirming previous studies,6,7 myeloid rDC that resemble pre-DC (analogous to the globular shape of resident plasmacytoid DC15) are also present at low density within the mesangium of CX3CR1<sup>GFP</sup>/+ mice.19 Importantly, recent studies indicated that a similar anatomic surveillance network of rDC exists throughout the interstitium and mesangium of normal human kidneys.21 Therefore, at steady state, rDC are positioned to respond immediately to “danger” or “tolerogenic” signals anywhere within the renal parenchyma, whether derived from self or nonself sources.

**ORIGINS AND MIGRATION OF RDC AT STEADY STATE**

Similar to DC in other nonlymphoid organs,13–35 rDC constitute a heterogeneous population of bone marrow–derived hematopoietic cells that, unlike DC that function solely within lymphoid tissues (most of the DC in the thymus and spleen and approximately half of the DC in lymph nodes), serve as sentinels within the kidney before trafficking to lymphoid organs.13–18 Because of the plasticity of FLT3<sup>+</sup> common myeloid and common lymphoid DC precursors to generate all DC subsets,16 local trophic cues rather than the availability of DC precursors probably determine the constitutuity of rDC at steady state. Notably, although administration of the ligand for FLT3 to mice can mobilize rDC precursors and transiently increase the total number of rDC, as it does for DC in other tissues,36 the proportion of each rDC subset is not significantly altered when compared with the normal steady state.10

Recent studies of myeloid rDC suggest a probable role of the renal microenvironment in stipulating the heterogeneity of rDC. Deriving from a shared clonogenic progenitor of the mononuclear phagocyte system37 along the long-lived “noninflammatory” CX3CR1<sup>high</sup>CCR2<sup>−</sup> monocyte lineage (also Ly6C<sup>+</sup> GR1<sup>+</sup> in mice; CD14<sup>+</sup>CD16<sup>+</sup> in humans),19,38–41 myeloid rDC can express markers conventionally assigned to macrophages (F4/80, CD68),10–12,19,42 This phenotypic overlap between myeloid rDC and macrophages20 is differential within normal kidneys; stellate-shaped CD11c<sup>+</sup> F4/80<sup>+</sup> rDC, for example, predominate in the medulla, whereas globular-shaped MHC class II<sup>+</sup> pre-rDC reside in the mesangium.6,7,19,43 This finding suggests that regional inductive factors shape the repertoire of rDC. It is interesting that engagement of the CSF-1 receptor is required for the differentiation and survival of myeloid DC,44 and constitutive expression of CSF-1 (i.e., M-CSF, a major chemoattractant and growth factor for mononuclear phagocytes45) by renal epithelial cells seems to be more prominent in the medulla than in the cortex at steady state.46,47 Whether this alone explains the heterogeneity of rDC is unclear. More likely, other differentially expressed trophic ligands (thymic stromal lymphopoietin-like factors that may be elaborated by renal epithelial cells or interstitial stromal cell populations48,49) and extracellular matrices within the kidney also contribute to the specification of rDC.

Trafficking rDC precursors express several chemokine receptors (CCR)50–53 that are also involved in establishing and maintaining the rDC network. Circulating immature mouse DC mobilized by FLT3 ligand migrate in vitro to CCR ligands expressed at basal levels within normal kidneys and, after adoptive transfer, traffic into the renal interstitium and mesangium in vivo.54 In contrast, isolated immature mouse rDC fail to demonstrate similar migration behavior despite expression of the same CCR.53 This suggests that renal-derived chemokines recruit trafficking rDC precursors to steady state, but once in residence, differentiating rDC precursors undergo CCR desensitization, possibly by molecules other than chemokines.50,53–55 In addition, the lack of redundant cellularity within the contiguous rDC network19 suggests exquisite, reciprocal downregulation or sequestration of chemoattractants directly at sites of occupancy by individual rDC.50

It is intriguing that self-renewal of rDC within the kidney may also occur (analogous to DC in the skin56 and ovaries57), a possibility hinted at by the small population (<3%) of resident CX3CR1<sup>high</sup>GR1<sup>−</sup> leukocytes in normal mouse kidneys that lack phenotypic

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*Expressed by some rDC in this subset.*
Figure 1. Surveillance of the renal parenchyma by the rDC network. The three panels shown are high-power views of the same location in the outer medulla of a normal kidney from a CXCR1GFP/+ mouse captured by confocal microscopy. The resident rDC network (A) is an anatomically separate cellular compartment from the renal parenchyma that it surveys (B). This affords rDC the ability to respond rapidly to local environmental cues, to migrate apart from nephrons, and to communicate with other components of the immune system both within and outside the kidney. (C) Merge of A and B demonstrates the intimate relationship that exists between rDC and the renal parenchyma.
markers of differentiated DC. Because preliminary bromodeoxyuridine-labeling experiments in normal mice suggest an average half-life across all rDC subsets of approximately 35 d (Matthew Griffin, personal communication; Matthew Griffin, MD Associate Professor Mayo Clinic, Rochester, Minnesota, USA, Date of communication: 2/22/07), this latter possibility, if true, will require innovative long-term studies to capture. Approaches such as total body irradiation, long known to be insufficient at fully ablating rDC, followed by bone marrow transplantation to reform the rDC network does not recapitulate normal rDC homeostasis and can lead to chimerism between donor and recipient rDC.

**FUNCTION OF RDC**

**Innate Immunity**
As sentinels of the immune system, rDC are positioned to integrate environmental cues and influence innate immune responses anywhere within the kidney. This functional plasticity rests in the intrinsic ability of tissue-resident, surveying DC such as immature rDC to sense and sample continually their immediate environment. This surveillance by rDC occurs by multiple mechanisms and includes the following: Engagement of a broad repertoire of surface receptors such as Toll-like receptors, receptors for alarmins, complement receptors, scavenger receptors, and cytokine and CCR; contact or paracrine-mediated signals from trafficking innate immune cells such as neutrophils, natural killer cells, natural killer T lymphocytes, and γδ T lymphocytes; contact or paracrine-mediated signals from trafficking adaptive immune cells; and contact (e.g., via tunneling nanotubes or paracrine-mediated signals from adjacent resident rDC).

In turn, the rapid integration of these external stimuli by rDC and their subsequent proinflammatory activation (if the stimuli are not tolerogenic) can prime and amplify innate immune responses. This occurs mainly through the secretion of cytokines (e.g., TNF-α, IFN-γ, IL-1β, IL-6, IL-12, IL-15) and chemokines by rDC that act on adjacent renal parenchymal cells and innate immune cells, which includes resident plasmacytoid DC as “professional type I IFN-producing” leukocytes. For example, resident myeloid rDC were recently identified as the predominant source of intrarenal TNF-α produced during the early “sterile” innate immune response to renal ischemia-reperfusion injury, an insult that also induces the maturation and migration of these resident myeloid rDC to lymphoid tissues. This initial cytokine response by resident rDC is separate from the cytokines subsequently released by recruited “inflammatory” CXCR1/CCR2 rDC precursors (also Ly6C, GR1+ in mice; CD14/CD16 in humans). These latter cytokines contribute to sequeleae of renal ischemia-reperfusion injury such as interstitial fibrosis, analogous to the cytokine response and egress versus influx of rDC versus rDC precursors, respectively, in response to LPS.

**Adaptive Immunity**
Not unexpected, rDC exhibit the hallmark of well-equipped antigen-presenting cells of the adaptive immune system. Resident rDC at steady state express MHC class I and class II molecules but bear low levels of co-stimulatory molecules (e.g., CD80, CD86, CD40), which are expressed on “professional type I IFN–producing” leukocytes. For example, resident myeloid rDC were recently identified as the predominant source of intrarenal TNF-α produced during the early “sterile” innate immune response to renal ischemia-reperfusion injury, an insult that also induces the maturation and migration of these resident myeloid rDC to lymphoid tissues. This initial cytokine response by resident rDC is separate from the cytokines subsequently released by recruited “inflammatory” CXCR1/CCR2 rDC precursors (also Ly6C, GR1+ in mice; CD14/CD16 in humans). These latter cytokines contribute to sequeleae of renal ischemia-reperfusion injury such as interstitial fibrosis, analogous to the cytokine response and egress versus influx of rDC versus rDC precursors, respectively, in response to LPS.

**Self-Tolerance**
DC in peripheral tissues contribute to self-tolerance by supplementing central tolerance. Preliminary studies indicate that rDC also play an important role in maintaining self-tolerance within the kidney. Immature rDC induce the expansion of T regulatory lymphocytes (Treg) in mixed leukocyte reactions and suppress allorecognition in vivo. These findings suggest that rDC may be tolerogenic if engaged by T lymphocytes that have escaped thymic selection and are reactive to self-antigens within the kidney. Moreover, cross-presentation of self-antigens derived within the kidney by rDC deletes autoreactive T lymphocytes in vivo by a Fas-mediated mechanism. These observations are intriguing because several tissue-specific self-antigens demonstrate low or no expression in the thymus and, thus, rely on peripheral mechanisms to help maintain tolerance. This is a potential confounder of many experimental kidney-restricted promoter systems that is often ignored.

Please note that the provided text is a natural representation of the image content, and it is not a direct transcription of a table or diagram.
For example, failure to regulate or delete autoreactive T lymphocytes generated during accelerated apoptosis of renal parenchyma, an instigator of autoimmunity,101,102 may explain why CD8+ T lymphocytes directed against kidney antigens, presumably presented by rDC, can develop in kdd/kdd mice.103,104

The ability of DC to process and present antigen can also be suppressed after contact with Treg,105,106 raising the possibility that resident rDC themselves are targets for trafficking Treg. Notably, Treg ameliorate both the acute and chronic phases of renal disease in disparate models of immune-mediated renal injury,107–109 although rDC-Treg interactions have not been studied in these experiments. This possible interaction is an exciting area of future inquiry, as is the potential to manipulate the reciprocal relationship between rDC and Treg to suppress immune responses that may be coordinated by rDC.110

### Immunologic Homeostasis of the Renal Parenchyma

Surveillance by DC resident in tissues may mediate self-tolerance to and regeneration of dying parenchymal cells.111,112 There is mounting evidence that the rDC network may fulfill this crucial role within the kidney. In mice, “alternatively” activated mononuclear phagocytes (i.e., an anti-inflammatory togeretic state in contrast to the “classically” activated proinflammatory state)113 clear apoptotic cells within the developing kidney as early as embryonic day 12, and challenge of embryonic kidney explants with M-CSF rapidly accelerates ureteric branching and nephron induction114 (David Hume, personal communication; David Hume, PhD Professor University of Queensland, Brisbane, Australia, Date of communication: 2/20/07). This suggests the establishment of mechanisms of apoptotic clearance during nephrogenesis that are coupled to paracrine growth loops115,116 between rDC and the renal parenchyma that persist in the mature kidney. It is interesting that hepatocyte growth factor, an important growth factor for renal epithelial cells,117 also alternatively activates DC,118 and DC that are matured in the presence of anti-inflammatory factors selectively express angiogenic isoforms of vascular endothelial growth factor.119 Although preliminary, these studies collectively suggest that rDC may participate in a coordinated program to control growth and maintain the homeostatic function of the renal parenchyma.

### CONCLUSIONS

In this brief review, we have discussed the current knowledge surrounding rDC. rDC function in the fundamental immunologic paradigm of continual surveillance. This is evidenced by the anatomic availability and intrinsic ability of rDC to respond rapidly to environmental cues anywhere within the kidney. rDC migrate and communicate these cues to both arms of the immune system and to the renal parenchyma and renew to maintain a contiguous network. In many respects, our understanding of rDC in the health and disease of the kidney is very rudimentary, spawned in part by the considerable lag in research on rDC compared with DC in other organs and tissues. Fortunately, much is now known about the biology of DC in general. Application of this knowledge should yield rapid and important advances in deciphering the contribution of rDC to the pathogenesis, such as their role in HIV infection in the kidney,120,121 and treatment, such as their targeting by CDK/GSK-3 inhibitors,122,123 of many renal diseases. This inquiry promises to be revealing in the years immediately ahead.

### ACKNOWLEDGMENTS

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### DISCLOSURES

None.

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