The Renal Lymph Node and Immune Tolerance to Filtered Antigens

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The past decade has seen remarkable progress in our understanding of the immunologic responses associated with diseases of the kidney. In particular, the diversity of bone marrow-derived immune cells present in the kidney during health and disease has been detailed by many laboratories using animal models and human biospecimens. As a result, we can now clearly distinguish the major classes of intrarenal immune effector cells (mononuclear phagocytes, polymorphonuclear leukocytes, T cells, B cells, and innate lymphocytes) as well as relevant subclasses of each—for instance, monocytes, macrophages, and dendritic cells (DCs) within the mononuclear phagocyte family.[1] Additionally, small animal-based studies have further subclassified immune cell types within the kidney based on their finer phenotypic details and functional characteristics—a good example being the various effector programs that predominate among intrarenal $CD4^+$ helper T cells (Th1, Th2, Th17, and regulatory) during different disease processes.[2]

A key element of recent progress has been the incorporation of emerging concepts and knowhow from the field of basic immunology into renal research. Notably, techniques such as multicolor flow cytometry, bone marrow chimerism, model antigen systems, adoptive cell transfer, and cell type-specific knockout and transgenic mice have opened the door to detailed dissections of intrarenal immunologic responses in the context of disease models.[1]

To a degree, however, our progress in renal immunology has been uneven, with a heavy emphasis on certain rodent models of AKI and acute GN as well as a strong focus on innate (nonantigen-specific) responses occurring within the kidney itself or adaptive (T cell and antibody) responses for which the antigen presentation pathways are unclear.[1] Although these preferences are understandable on the basis of the clinical importance of AKI and GN coupled with the availability of suitable models, there are some potentially fascinating areas that remain underexplored. Two such areas are the study of the kidney and its draining lymph node as an immunologic axis and the mechanisms by which immune tolerance is maintained to antigens that are either exclusively expressed by cells of the kidney or become concentrated within the renal interstitium as a result of glomerular filtration and reuptake.

In the current issue of *JASN*, a study from the laboratory of Christian Kurts elegantly addresses these two issues using a very precise set of experimental approaches from the mouse immunology toolbox.[3] In a simple but revealing first experiment, Gottschalk *et al.*[3] show that fluorescent ovalbumin is taken up within 10 minutes of intravenous injection by a substantially higher proportion of the DCs in renal lymph nodes (RLNs) than other lymph nodes. The timing of this observation, together with previously published work from the same group,[4] indicates that intravascular ovalbumin is filtered in the kidney and reabsorbed into the interstitium from where it is quickly carried by lymphatics to the RLN and internalized by DC populations resident there. Given the well known function of DCs to present antigen to resting T cells,[5] the most significant question to ask at this point was whether ovalbumin-loaded DCs are capable of activating T cells with specificity for this antigen within the RLN. Interestingly, when ovalbumin-specific $CD8^+$ (OT-1) T cells were introduced into the system, those cells that became localized to the RLN had an increased rate of apoptosis and decreased production of the signature cytokine IFNγ compared with T cells encountering ovalbumin under immunogenic conditions in subcutaneous lymph nodes. When OT-1 T cells were retrieved from the RLN after a primary exposure to DCs that had internalized filtered ovalbumin and then transferred into new recipients, they also proved to have very poor capacity to survive *in vivo* or produce IFNγ. Thus, with no additional experimentation, it could be concluded that, for this particular filtered antigen at least, DC-mediated presentation within the RLN results in two of the classic phenomena associated with peripheral immune tolerance: clonal deletion and antigen-specific hyporesponsiveness of $CD8^+$ cytotoxic T cells.[5] This intriguing finding raises secondary considerations regarding the specific signals and RLN-resident DC subpopulations that are responsible for tolerogenic presentation of filtered antigen.

Using more complex experimental approaches, Gottschalk *et al.*[3] have also provided quite clear and convincing answers to

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these questions. In the first place, a role for the negative regulatory cell surface protein programmed death ligand 1 (PD-L1) was shown through the use of blocking antibodies and OT-1 cells derived from mice genetically lacking the PD-L1 binding partner PD-1. In the second place, a subpopulation of DCs that is characterized by the surface marker phenotype $CD11b^-/CD8^+/CD103^+/XCR1^+$ was shown to internalize filtered ovalbumin in the RLN, inherently express PD-L1, and play an essential role in tolerizing OT-1 cells by PD-L1. In proving the latter point, Gottschalk *et al.*[3] took advantage of the observation that the protein basic leucine zipper transcription factor, activating transcription factor-like 3 (Batf3), is required for development of crosspriming $CD8^+$ DCs.[6] In bone marrow chimera experiments involving mixtures of Batf3-deficient and PD-L1–deficient donor marrow transferred into wild-type or PD-L1–deficient recipients, Gottschalk *et al.*[3] convincingly show that neither $Batf3^+/PD\text{-}L1^-$ nor $Batf3^-/PD\text{-}L1^+$ DCs were sufficient to induce apoptosis of OT-1 cells encountering filtered ovalbumin within the RLN.

From an immunologic perspective, this excellent study by Gottschalk *et al.*[3] is both consistent with established details of DC-mediated crosspresentation and novel in that it identifies the kidney and its draining lymph node as a system in which these mechanisms are uniquely active in tolerizing the T cell compartment to small protein antigens during health. DCs in peripheral lymphoid and nonlymphoid tissues are known to contribute to self-tolerance by supplementing central (thymic) tolerance.[5] The specific phenomenon by which DCs capture extracellular foreign or autologous antigens and present their peptides on MHC-I to $CD8^+$ T cells in the periphery was first described as a mechanism for immune tolerance in mice in the 1970s.[7] Subsequently, it was shown that the mouse DC populations that possess specialized machinery for crosspresentation are $CD8^+$ in the lymphoid organs[8] and $CD103^+/CD11b^-$ in the nonlymphoid organs.[9] In 2008, Batf3 was identified as a key regulator of mouse $CD8^+$ DC development, because $Batf3^{-/-}$ mice exhibited impaired cross-presentation and ineffectual cytotoxic T cell responses against viral infection and tumors.[6] Very recently, splenic and peripheral Batf3-dependent DCs can be also defined by shared high expression of DNGR-1 (dendritic cell, natural killer cell lectin group receptor-1), a receptor for necrotic cells that is required by DCs to crossprime cytotoxic T cells against dead cell antigens.[10] Moreover, DNGR-1 is a unique marker of a human Batf3-expressing $CD11b^-$ DC population present in both lymphoid and nonlymphoid tissues.[10] Thus, the remarkable progress that has been made in delineating the molecular details of DC-mediated peripheral tolerance in mouse is providing important clues to the mechanisms underlying similar processes in humans.

As the experiments of Gottschalk *et al.*[3] show, circulating antigens may induce a form of peripheral immune tolerance that is sustained through clonal deletion and/or inactivation of lymphocytes.[3] Their results support a model, whereby binding of PD-1 on the T cell by PD-L1 expressed by the presenting DC during primary antigen encounter mediates proapoptotic and proanergic intracellular signaling within the T cell. However, because a proportion of the OT-1 cells in the RLN remained viable and capable of IFNγ production, it is possible that other mechanisms also contribute to immune tolerance to filtered and kidney-specific antigens. For instance, Kurts *et al.*[11] have previously identified a role for CD95 (Fas) in the peripheral deletion of OT-1 T cells in a model involving crosspresentation of a transgenic, membrane-bound form of ovalbumin expressed in the kidney and pancreatic islets. These studies highlight the fact that there is much left to learn regarding the mechanistic basis for immune tolerance to the protein antigens that are specifically associated with the kidney and its filtering functions. Furthermore, because experiments involving OT-1 cell adoptive transfer create isolated $CD8^+$ T cell responses, it will now be interesting to examine how coincident $CD4^+$ and $CD8^+$ T cells responses to filtered antigens are regulated within the kidney/RLN axis. In this regard, Edgtton *et al.*[12] have previously reported a role for PD-1/PD-L1 interactions in limiting the proliferation of ovalbumin-specific $CD4^+$ T cells in the RLN.

Clearly, it is important to consider what relevance this study may hold for human health. Although most of the experimental strategies carried out by Gottschalk *et al.*[3] in the mouse could not be conducted in human subjects, it is highly likely that transfer of filtered small proteins to RLN-resident DCs is a generalizable phenomenon. Furthermore, the key components of the immune tolerance mechanisms identified in the study—DC subpopulations with specialized capacity for crosspresentation and negative regulatory signaling by PD-L1/PD-1 interactions—are known to be applicable to the human immune system.[13,14]

One relevant question to ask, therefore, is whether tumor antigen-specific cytotoxic T cells are deleted or rendered hyporesponsive in the RLN of human subjects with renal cancers or nonrenal neoplasms that shed low-molecular weight antigens into the bloodstream. Because interventions to modulate DC-mediated tumor antigen crosspresentation and block tolerogenic costimulatory pathways in cancer patients are now gaining credence in the clinical arena,[14,15] this question represents more than just an intellectual challenge and merits additional attention in experimental models. Similarly, we may also ask whether intrarenal inflammation, such as occurs in many forms of AKI and CKD, results in the loss of peripheral immune tolerance to filtered and kidney-specific antigens through its modifying effects on crosspresenting DCs in the RLN. Although there is some experimental evidence for an autoimmune component to inflammatory renal injury,[16] this interesting possibility has not been studied in great detail to date.

It is to be hoped that the compelling findings that Gottschalk *et al.*[3] have generated by applying state-of-the-art immunologic approaches to filtered antigen crosspresentation in the RLN will stimulate interest in addressing other unanswered questions about the immunologic dynamics of the kidney and its draining lymph node during health and disease.

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DISCLOSURES

None.

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See related article, "Batf3-Dependent Dendritic Cells in the Renal Lymph Node Induce Tolerance against Circulating Antigens," on pages 543–549.

Getting the "Inside" Scoop on EphrinB2 Signaling in Pericytes and the Effect on Peritubular Capillary Stability

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Progressive CKD develops from a lack of fidelity in tissue remodeling after acute injury. A lack of successful repair following even subtle injuries, regardless of their cause, suggests that complete resolution of function may be difficult to achieve, even if initiating deficits are corrected. This pathologic repair further burdens the adaptive capacity of the kidney and contributes to steady decline in function.[1] Interstitial fibrosis is the commonly recognized histologic feature of this pathologic remodeling,[1] which is present in all models of CKD, and is highly correlated with progression to ESRD.

The last 10–15 years have witnessed the articulation of new hypotheses on the development of interstitial renal fibrosis. For example, it is now clear that all forms of CKD are associated with a reduction of peritubular capillaries, which results in exacerbation of hypoxia and the activation of molecular pathways associated with scar formation.[2] The recognition that capillary rarefaction precedes fibrosis in models of AKI indicates that capillary loss is not solely attributable to encroaching fibrosis but rather may initiate or contribute to the fibrosis.[2] Despite the presence of a hypoxic environment that should support angiogenesis, it is unclear why the kidney capillaries seem incapable of mounting an effective repair response.

In addition to an increased interest in capillaries, several theories have been promulgated regarding the nature and

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