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Cross Dendritic Cells Anger T Cells after Kidney Injury

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Nearly 30 yr ago, Stewart Cameron caused a minor revolution by pointing out that simple quantitation of proteinuria was much better at predicting outcome than histopathological diagnosis—then the gold standard prognostic marker.[1] We now know that severe proteinuria does not simply reflect glomerular injury but is itself harmful.[2] Reabsorption of large amounts of protein from the glomerular filtrate induces stress responses in proximal tubular epithelial cells that result in lysosomal instability and can even initiate epithelial-mesenchymal transition.[3] Epithelial cells synthesize chemokines, cytokines, and complement components that recruit inflammatory cells and lymphocytes into the interstitium causing progressive fibrosis. A fascinating paper by Macconi *et al.* from Ariela Benigni's group in the current issue of JASN[4] adds another layer of complexity by showing that albumin reabsorbed from proximal tubules induces the generation of albumin-specific, IFN-γ secreting, CD8 T cells that may contribute to progressive renal injury.

Before discussing the results in detail, it is important to consider how proteins reabsorbed by the proximal tubule are presented to T cells. The interstitium of normal kidneys contain numerous resident monocytic myelocytes, traditionally believed to be macrophages because they express relevant markers such as F4/80 in mice and CD68 in man.[5] It is now known they also express dendritic cell markers and can indeed present antigen—one of the defining features of renal dendritic cells with broadly similar characteristics to dendritic cells in other tissues.[5–10] Studies in mice with fluorescently labeled dendritic cells show essentially all resident myeloid cells are dendritic, reveal their intimate connection with renal tubules through long processes kissing the epithelial cells, and thus are ideally positioned to sample reabsorbed antigens or receive activation signals from them.[6,7] Consequently, renal dendritic cells are the likely sentinals that sample renal antigens and present them to T cells. The endocytosed antigen is processed in one of two ways: either in endocytic compartments to create peptides that are loaded on to MHC class II molecules that

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present to $CD4^+$ T helper and T regulatory cells,[11] or, alternatively, they are processed by a different pathway involving proteosomal degradation that loads MHC class I molecules for presentation to $CD8^+$ T cells.[12] This latter pathway is known as "cross-presentation" and is the exclusive focus of the work considered here.

Macconi *et al.* investigate how reabsorbed albumin interacts with the immune system.[4] First they show that albumin endocytosed by rat proximal tubular cells *in vitro* is digested to a discrete set of peptides that are released into the supernatants of cultured cells—the predominant peptide being the aminoterminus fragment, albumin 1 to 24. Next, they demonstrate that albumin 1 to 24 is endocytosed by rat dendritic cells and digested by a proteasome-dependent pathway to 8- to 10-mer peptides that are loaded onto MHC class I molecules and presented to $CD8^+$ T cells. The first encounter between albumin 1- to 24-pulsed renal dendritic cells primes naive $CD8^+$ T cells, so that when exposed a second time, CD8 cells become activated and secrete IFN-γ. The authors then went on to prove this was not an *in vitro* artifact with an experimental tour-de-force using T cells and dendritic cells harvested from kidneys and draining lymph nodes of rats made proteinuric by 5/6 nephrectomy. They demonstrate 4 wk after nephrectomy that CD8 T cells purified from renal lymph nodes produce IFN-γ when incubated with renal dendritic cells pulsed with albumin 1 to 24—suggesting they were educated *in vivo* during the course of the disease. This suggestion was confirmed by further experiments showing that $CD8^+$ T cells harvested in the same way 1 wk after nephrectomy do not respond when first cultured with albumin-pulsed dendritic cells but behave like the naive $CD8^+$ T cells in the *in vitro* studies; that is, they need priming to produce an albumin 1- to 24-specific IFN-γ response. Similarly, *in vivo* treatment with the proteosome inhibitor, bortezomib, which prevents MHC class I loading with antigen, prevents CD8 education *in vivo*. Taken together, these results provide a compelling case that rats develop IFN-γ-secreting $CD8^+$ (presumptively cytotoxic) T cell responses to albumin after 5/6 nephrectomy.

The results from the Begnini group present an interesting contrast with a paper published earlier this year, from Christian Kurts' group.[13] The approach was very similar except the latter experiments were performed in normal mice and the antigen examined, ovalbumin, was small enough to be freely filtered by the glomerulus. As expected, administered ovalbumin concentrates in the kidney, principally in proximal tubules, and transfers to dendritic cells in the kidney and the renal lymph nodes. Here it is cross-presented to $CD8^+$ T cells that react by proliferating when pulsed with ovalbumin. However, the outcome contrasted with the Macconi studies in that the responding T cells did not produce IFN-γ or become cytotoxic but instead became apoptotic.

Accordingly, both studies demonstrate that filtered antigen concentrated and reabsorbed by the proximal tubular epithelium is cross-presented to $CD8^+$ T cells by renal dendritic cells or dendritic cells in the draining lymph node. However, both studies suggest the T cell response depends on context. Cross-presentation by dendritic cells from normal kidneys reinforces immunological tolerance to the filtered antigen whereas cross-presentation by dendritic cells from a proteinuric kidney results in an active immune response. This idea is consistent with data on cross-presentation in other contexts: these other contexts show that for cross-presentation to result in active immunity, the presenting dendritic cells must be exposed to endogenous signals released from dead or damaged cells—so called "damage-associated molecular patterns" (DAMPs).[14]

Much more needs to be known before the role of albumin-specific T cells can be clinically assessed in chronic kidney disease. Obviously the critical question is whether these albumin-specific, IFN-γ-producing $CD8^+$ T cells cause injury—an issue not addressed in the paper presented here. For example, do $CD8^+$ T cells kill albumin-pulsed renal epithelial cells? They also raise issues about the role of the epithelial cells themselves—is processing to albumin 1 to 24 essential for the response of dendritic cells or is intact albumin equally good at generating CD8 primed T cells? Do the epithelial cells express the DAMPs, and if so, what are the critical molecules and which dendritic cell receptors do they ligate? Answers to these and other questions are bound to be forthcoming very shortly.

In conclusion, the demonstration of a highway for rapidly concentrating small protein molecules and delivering them directly into the renal interstitium or lymph nodes raises the wealth of exciting therapeutic possibilities for understanding and managing progressive renal injury.

DISCLOSURES

None.

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See related article, "Proteasomal Processing of Albumin by Renal Dendritic Cells Generates Antigenic Peptides," on pages 123–130.

Salt in the Wound

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Aldosterone activates the epithelial sodium channel (ENaC) in principal cells of the collecting duct to regulate salt excretion, extracellular volume, and BP. Seminal studies by Hostetter and colleagues[1] demonstrated that aldosterone also contributes to glomerular sclerosis in a remnant kidney model. Treatment of rats with aldosterone and salt induces an inflammatory response, characterized by perivascular leukocyte infiltration and increased renal expression of osteopontin, monocyte chemoattractant protein (MCP-1), IL-6, and IL-1β through a mineralocorticoid receptor (MR)-dependent mechanism.[2] Not surprising, MR activation by aldosterone stimulates the expression of proinflammatory and profibrotic genes through an MR-dependent mechanism in cultured vascular cells, mesangial cells, podocytes, and fibroblasts.[3,4] In this issue of *JASN*, however, Leroy *et al.*[5] report that aldosterone also activates the canonical NF-κB pathway and proinflammatory genes in cultured principal cells, the site of MR-mediated sodium reabsorption and potassium excretion. Whereas in mesangial cells and vascular smooth muscle cells, aldosterone activates p38 and extracellular signal–regulated kinases 1 and 2,[6,7] aldosterone stimulates NF-κB in principal cells through an MR-dependent increase in serum and glucocorticoid-induced kinase 1 (SGK1) expression but not through extracellular signal–regulated kinase or p38.

Leroy *et al.* also observed *in vivo* in rats that dietary salt restriction, associated with increased circulating concentrations of aldosterone, increases MR-dependent expression of mRNA encoding NF-κB and SGK1 in the cortical collecting duct. Whether activation of the glucocorticoid receptor *in vivo* would dampen the proinflammatory effects of MR activation under pathophysiologic conditions in which both aldosterone and cortisol (or corticosterone) are elevated, as glucocorticoid receptor activation did *in vitro*, was not specifically addressed.

What is the physiologic relevance of this convergence of salt reabsorption and inflammatory stimulation in the principal cell? It is possible that, during low salt intake, increased inflammation puts a break on sodium reabsorption. The same group previously reported that prolonged activation of NF-κB by LPS decreases expression of SGK1 and activity of the ENaC-α subunit, as well as basal, glucocorticoid, and mineralocorticoid-stimulated sodium transport in cultured principal cells.[8] Bens *et al.*[9] also reported that LPS reduced amiloride-sensitive ion fluxes in cultured cortical collecting duct cells. In addition, increased expression of the NF-κB target gene plasminogen activator inhibitor 1 (PAI-1) during low salt intake could decrease activation of ENaC by decreasing plasmin-mediated proteolytic cleavage of its γ subunit.[10]

Although Leroy *et al.* found that low salt intake increases MR-dependent activation of NF-κB in the collecting duct, it is well established that *high* salt intake enables the proinflammatory and profibrotic effects of aldosterone in the kidney in whole-animal studies. High salt intake paradoxically activates renal MR in obese hypertensive rats, resulting in increased translocation of the MR to the nucleus, increased expression of SGK1, and increased NF-κB activity.[11] Although the cell specificity of this effect is not known, MR antagonism decreases renal NF-κB expression and glomerular podocyte injury in parallel during high salt intake. Taken together with the observation that low salt intake increases MR-dependent inflammation in the cortical collecting duct, it is possible that salt intake modulates the site and proinflammatory effect of aldosterone in the kidney. Increased oxidant stress also contributes to salt-induced activation of renal MR, in that the antioxidant tempol prevents the effect. Likewise, aldosterone increases PAI-1 expression in mesangial cells in part through increased oxidative stress and TGF-β.[4] It would be interesting to know whether increased oxidative stress contributes to the MR-dependent activation of NF-κB in the cortical collecting duct during low salt intake.

Aldosterone or MR activation causes both tubulointerstitial fibrosis and glomerulosclerosis in animal models. Thus, the MR antagonists spironolactone and eplerenone decrease interstitial inflammation and glomerular injury in rats with radia-

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