8. Hatherley D, Lea SM, Johnson S, et al. Polymorphisms in the human inhibitory signal-regulatory protein alpha do not affect binding to its ligand CD47. *J Biol Chem*. 2014;289:10024–10028.

9. Rodriguez PL, Harada T, Christian DA, et al. Minimal "Self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. *Science*. 2013;339:971–975.

translational science

A novel role for renal epithelial cells and the medullary sodium gradient in the local immune response

Christina M. Wyatt[1] and Steven D. Crowley[1,2]

The extreme hypertonicity of the renal medulla plays a central role in regulating volume status. A recent publication in *Cell* has identified a novel role for the high sodium environment and the local epithelial cells in the recruitment of mononuclear phagocytes, potentially contributing to the defense against ascending urinary tract infection.

Refers to: Berry MR, Mathews RJ, Ferdinand JR, et al. Renal sodium gradient orchestrates a dynamic antibacterial defense zone. *Cell*. 2017;170:1–15.

Kidney International (2017) **92,** 1308–1311; http://dx.doi.org/10.1016/j.kint.2017.10.005

KEYWORDS: chemokine; cytokines; diabetes insipidus; macrophages; pyelonephritis

Epithelial cells have a well-established role as the first line of defense against infection through barrier functions in the skin and mucosal surfaces. More recently, epithelial cells in the skin and the gastrointestinal and respiratory tracts have also been shown to respond to environmental stimuli to trigger local cellular immune responses. In a recent study, Berry *et al.*[1] demonstrated a novel role for renal epithelial cells in the recruitment of mononuclear phagocytes to the renal medulla in response to the high sodium environment.

Initial studies in human kidney tissue identified a population of macrophage-like CD14+ mononuclear phagocytes that were disproportionately represented in the renal medulla. The authors also observed differential expression of chemokines known to promote migration of mononuclear phagocytes, with higher expression of CCL2 and CX3CL1 in renal medullary *versus* cortical tissue. The authors hypothesized that the unique high sodium environment of the renal medulla may serve as a stimulus for epithelial cell chemokine production. Supplementation of culture medium with sodium chloride (NaCl) to mimic the sodium content of the renal medulla stimulated the production of CCL2 and CX3CL1 by immortalized human tubular epithelial cells. Chemokine production was slightly more pronounced following exposure to both high sodium medium and uropathogenic *Escherichia coli* (*E. coli*), but did not occur following treatment with mannitol as a hyperosmotic control. The authors identified the transcription factor nuclear factor of activated T cells 5 (NFAT5) as a potential mediator of the epithelial cell response to the high sodium environment, as previously demonstrated in other tissues.[2,3] Expression of *NFAT5* was increased in human renal medullary *versus* cortical tissue, and knockdown of *NFAT5* or inhibition of kinases required for its activation in the tubular epithelial cell line attenuated chemokine production in response to NaCl.[1]

To confirm that the differential localization of CD14+ mononuclear phagocytes is dependent on the medullary sodium gradient *in vivo*, the authors treated mice with tolvaptan or demeclocycline to induce diabetes insipidus (DI). In contrast to control mice, there was a loss of the differential distribution of CD14+ cells and the differential expression of *CCL2* and *NFAT5* between the medulla and cortex in treated mice. Similar findings were observed in kidney tissue from deceased kidney donors with evidence of central DI. Neutralization of CCL2 or genetic deletion of its receptor or *NFAT5* in untreated

[1]*Division of Nephrology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA; and* [2]*Division of Nephrology, Department of Medicine, Duke University and Durham VA Medical Centers, Durham, North Carolina, USA*
Correspondence: *Steven Crowley, Duke University School of Medicine, 2nd Floor, Room 2018, MSRB II, 106 Research Drive, Durham, North Carolina 27710, USA. E-mail: steven.d.crowley@duke.edu*

mice had a similar effect on the distribution of CD14+ mononuclear cells. Based on further *in vivo* experiments, the authors concluded that the enrichment of CD14+ mononuclear phagocytes in the renal medulla reflects recruitment of circulating monocytes to the medulla in response to CCL2 release rather than local proliferation.[1]

In vitro, CD14+ mononuclear phagocytes isolated from human renal medullary tissue displayed increased phagocytic activity and increased cytokine production compared with CD14− mononuclear cells. This response was enhanced in the presence of *E. coli*, suggesting a potential role in defense against ascending urinary tract infection (UTI). CD14+ mononuclear phagocytes also demonstrated greater phagocytic activity, cytokine production, and dendrite extension when grown in high sodium culture conditions, suggesting that the high sodium environment of the renal medulla may promote both the recruitment and function of these cells. *In vivo*, mice with drug-induced DI had more renal abscess formation and higher rates of sepsis and death following intravesicular challenge with *E. coli*. More severe ascending infections were also observed with inhibition of CCL2 or genetic deletion of its receptor or *NFAT5* in mice.[1]

These data support the authors' hypothesis that the high sodium environment of the renal medulla may serve a secondary purpose, promoting the recruitment and function of mononuclear phagocytes to defend against ascending UTI. The authors propose an elegant but unproven model whereby the recruitment of antibacterial phagocytes is enhanced in the setting of volume depletion when the medullary sodium concentration is increased and urinary flow rates are decreased. Because high urinary flow rates are thought to provide a mechanical barrier to ascending infection, the heightened immune response could theoretically compensate for the loss of this protective mechanism. Of note, the limited human data presented in support of this model do not clearly distinguish between ascending and lower genitourinary tract infections. In human deceased kidney donors with evidence of central DI, the authors observed a higher rate of bacterial growth in ureteral cultures compared with deceased donors without evidence of DI, but data on parenchymal infection were not obtained. The authors also pointed to published reports of increased UTIs in human subjects receiving tolvaptan for autosomal dominant polycystic kidney disease and in patients with sickle cell disease; however, it is likely that the majority of those infections were lower UTIs that may or may not be affected by the local immune environment in the renal medulla.[1]

Nonetheless, the authors have assembled a compelling dataset suggesting that hypertonicity in the renal medulla may afford protection from ascending UTI by enhancing the proinflammatory actions of intrarenal myeloid cells (Figure 1). These data are consistent with the notion that myeloid cells within different regions of the kidney play distinct roles in the innate immune response. The Kurts group previously demonstrated that CX3CR1-expressing myeloid cells in the kidney cortex play a role in autoimmune glomerular disease, whereas these same cells in the medulla limit the severity of pyelonephritis.[4] The findings of Berry *et al.* suggest that a hypertonic environment in the renal medulla may have enhanced the capacity of the medullary CX3CR1-expressing cells to confront bacterial infection.

Together with other published data, these findings support a paradigm of bidirectional feedback between resident tissue myeloid cells and mucosal NaCl concentrations, integrated through the tonicity sensor NFAT5. Salt in the interstitial spaces of epithelial barrier tissues primes innate and adaptive immune cells to ward off invading microbes. In the renal medulla, hypertonicity primes interstitial mononuclear phagocytes to prevent pyelonephritis, while at sites of bacterial or protozoan infection in the skin, salt accumulates and activates resident macrophages, also through an NFAT5-dependent mechanism.[2] NaCl has also been shown to directly influence the adaptive immune response, promoting proinflammatory polarization of T lymphocytes toward a Th17 phenotype known to be critical for mucosal immunity.[5]

There are several costs of salt-driven immune activation. First, inappropriate stimulation of proinflammatory macrophages and T cells in the absence of infection has been shown to provoke autoimmunity.[5] Second, mucosal hypertonicity may suppress the functions of alternatively activated macrophages that typically participate in wound repair, as previously documented in the skin.[6] It would be interesting to know whether there are also secondary effects on the development of kidney fibrosis, particularly as the renin-angiotensin system is a primary driver of both sodium