
See related article, “Activated Protein C Ameliorates Renal Ischemia-Reperfusion Injury by Restricting Y-Box Binding Protein-1 Ubiquitination,” on pages 2789–2799.

Urine CXCL10/IP-10 Fingers Ongoing Antibody-Mediated Kidney Graft Rejection

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Location matters! The trafficking and positioning of cells within the innate and adaptive immune compartments are key features of the immune system that optimize the generation of responses to pathogens and to other inflammatory stimuli. This positioning is directed through the production of cytokines with chemoattractant functions, chemokines which direct the trafficking of the cells to a specific location.1–2 The approximately 50 chemokines are grouped into four families based on the presence of a cysteine-cysteine motif near the amino terminal end of the protein, with the C-C and CXC chemokines comprising most members of the superfamily. Chemokines exert their function through binding to seven transmembrane-spanning G-protein–coupled receptors, which transduce the signals mediating actin reorganization to direct target cell movement. There are approximately 20 different chemokine receptors that can be expressed to direct cell trafficking and positioning to specific tissue locations. The outnumbering of chemokine receptors by chemokines results in promiscuous relationships with many chemokine receptors binding multiple chemokines, although each chemokine ligand may transduce target cell–specific signaling and exhibit fidelity in function.

In addition to the positioning of immune and other cells during development, a primary function of chemokine–chemokine receptor interactions is to direct the trafficking of innate and antigen-specific immune cells to sites of inflammation, such as during an infection or allograft injury.1,2 Depending on the tissue, inflammatory signals will induce many different cells such as epithelial cells, endothelial cells, macrophages, neutrophils, and lymphocytes to produce sets of specific chemokines. A complementary feature of the adaptive immune system is that in the secondary lymphoid organs, T cells are induced to express chemokine receptors, facilitating their trafficking to and localization at specific tissue sites; the net consequence is optimization of the immune response to a particular insult. For example, T cells generated in response to a fungal or extracellular bacterial infection produce cytokine IL-17 and display the chemokine receptor CXCR6; this directs T cells to sites where the infection has stimulated the production of the chemokine CCL20. On the other hand, IFN-γ is needed in responding to intracellular bacterial infections. During these responses, antigen-specific CD4 and CD8 T cell development to effector T cells that produce IFN-γ requires gene transcription; such transcription is driven by T-bet, which also promotes the expression of the chemokine receptor CXCR3.3–4 The production of IFN-γ at the inflammatory site by memory T cells, natural killer cells and other innate cells stimulates the production of the IFN-γ–induced chemokines CXCL9/Mig, CXCL10/IP-10 and CXCL11/I-TAC; these chemokines, in turn, recruit CXCR3+ effector CD4 and CD8 T cells to the inflammatory site, thereby mediating the adaptive immune response. Importantly, these processes are operative during the response to allografts; in particular, the production of CXCL9 and CXCL10 play key roles in directing donor antigen–primed T cells into the allograft where they are activated to express the functions mediating acute and chronic graft injury.5,6

There has been considerable effort in identifying biomarkers that indicate ongoing T cell–mediated graft injury. For renal transplant patients, this has included the interrogation of mRNA isolated from for-cause biopsies and, importantly, as a noninvasive approach, the interrogation of protein and/or mRNA in urine using standard ELISA and newly developed approaches to quantitate mRNA in the urine sediment.7–12 Several of these studies have indicated that the presence of CXCL9 and/or CXCL10, assessed either by protein or mRNA levels, and determined either in the patients’ graft or in urine, strongly correlates with the Banff criteria diagnosis of T cell–mediated rejection. These chemokines are produced by a variety of renal graft cells, including endothelial and tubular epithelial cells and kidney resident macrophages and dendritic cells, as well as graft-infiltrating macrophages and neutrophils. As antibody-mediated rejection

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has garnered much attention recently, several groups have initiated studies to identify noninvasive biomarkers indicative of ongoing antibody-mediated acute injury.

In an original study reported in the current issue of the Journal, Rabant and coworkers have identified for the first time the intriguing association between CXCL10 protein levels in the urine with the diagnosis of ongoing antibody-mediated rejection in the human renal allograft. The authors further show that CXCL10 levels increase during antibody-mediated rejection that is accompanied by microvascular inflammation; in contrast, CXCL9 levels increase during T cell–mediated rejection that is accompanied by tubulointerstitial inflammation. This distinction raises interesting questions about the nature of inflammatory factors stimulating these distinct patterns of CXCR3 ligand chemokine production and the accompanying renal graft histopathologies. The production of CXCL9 is more stringently dependent on IFN-γ stimulation, whereas CXCL10 production is also stimulated by type I IFNs and by toll-like receptor agonists that activate NF-kB translocation from the cytoplasm to the nucleus. On this basis, one could hypothesize that antibody-mediated injury generates the endogenous or sterile toll-like receptor ligands and that such generation fails to occur in T cell–mediated rejection. The results of the Rabant study also suggest that CXCL10 is a more sensitive indicator of the types of inflammation that may occur during antibody-mediated acute and chronic injury in renal grafts. While this study offers a potentially strong noninvasive biomarker to detect antibody-mediated injury in renal grafts, many more questions are raised by this investigation, as any good study would be expected to. These include: (1) do CXCL10 levels anticipate a future episode of antibody-mediated rejection when measured in sequential samples? (2) What is the correlation of CXCL10 levels in urine with donor-specific antibody (DSA) titers pretransplant and de novo produced DSA? (3) What is the influence of DSA specificity and isotype on the levels of CXCL10 detected in the urine? Furthermore, analyses were only conducted at single time points of renal dysfunction and suspected immune-mediated injury in the patient cohort. It is thus important to determine if urinary CXCL10 levels decrease to normal/near-normal levels following resolution of the antibody-mediated rejection episode, and whether such levels continue to increase in those patients whose grafts eventually fail. Another issue of significance is the need to distinguish antibody-mediated rejection from T cell–mediated rejection; the study by Matignon et al. suggests that this is indeed feasible.

What explains the finding that urine CXCL10 levels plus DSA are diagnostic and/or prognostic of antibody-mediated rejection while the presence of DSA alone is not? We may speculate that urine IP-10 is a functional readout for the ability of DSA to activate kidney resident endothelial cells or other cells and initiate the anti-allograft repertory.

The Rabant study and related observations advance CXCL10 and related chemokines as attractive targets to quench the anti-allograft response. Due caution, however, is required, because disruption of the CXCR3-chemokine axis has produced some unexpected results, such as enhanced rather than reduced autoimmunity, albeit in experimental models.

Where should the transplant community go from here? We believe that sufficient information exists that heightened expression of CXCL10 and related chemokines in urine, at the mRNA or protein level, is associated with ongoing acute rejection in the kidney allograft. We think it is time to put the diagnostic and/or prognostic biomarkers identified in this study and elsewhere to direct testing. In appropriately designed prospective clinical trials, we should investigate whether the detection of an inflammatory signature is a sufficient trigger for a “for-cause” biopsy despite normal allograft function; we should also investigate whether the absence of the inflammatory signature in the urine argues against the need for a surveillance biopsy. We note with concern the wasted efforts when 70%–80% of surveillance biopsies are classified as normal. In this regard, we believe that it would not only be cost-efficient but also enhance patient safety to investigate whether those with normal biopsies can be identified a priori by the absence of an inflammatory signature in urine.

Precision medicine is in vogue now, but transplant medicine has been a vanguard in selecting the best kidney recipient/donor combination by HLA matching and DSA screening. Individualized immunosuppressive therapy, rather than one-size-fits-all, is a worthwhile objective. Rabant et al. deserve credit for bringing to our attention that T cell–mediated rejection and antibody-mediated rejection are not separate universes, but share a signature that is eminently measurable in the urine samples from kidney transplant recipients.

DISCLOSURES

None.

REFERENCES


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C4d Staining in the Diagnosis of C3 Glomerulopathy

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In 2010 we suggested the term C3 glomerulopathy for glomerular disease in which there was isolated deposition of C3 in glomeruli in the absence of immunoglobulins and of components of the classic pathway of complement activation. The presence of such isolated C3 implies that complement has been activated via the alternative pathway and alerts the clinician to the need to investigate the complement system for genetic or acquired complement dysregulation. C3 glomerulopathy may have a range of appearances by light microscopy and electron microscopy. Many cases show a membranoproliferative pattern but some are mesangial proliferative and some show prominent endocapillary hypercellularity emphasizing the importance of making a diagnosis based on pathogenesis rather than pure morphology. On electron microscopy, cases can be divided into those which show very osmiophilic dense transformation of the GBM (dense deposit disease) and those without that appearance which have been designated C3 glomerulonephritis. Although the original concept was based on the presence of isolated C3 it is clear that, in practice, this is too stringent a definition and that, if cases where the underlying pathology is alternative pathway activation are not to be missed, a wider net is needed. This was clearly shown by a study from Hou et al. They looked at cases of dense deposit disease in which it is generally accepted that the glomerular changes result from abnormal control of the alternative pathway of complement. They found that if they used a stringent criterion of only C3 with no immunoglobulins then 50% of cases diagnosed as dense deposit disease on electron microscopy would not be classified as C3 glomerulopathy. In order to identify the great majority of cases in which the patient requires investigation of the complement pathway they suggested that the best cut-off would be dominant C3 with C3 staining at least two orders of magnitude more intense than any other component. In this issue of JASN, Sethi and colleagues propose that staining for C4d may be useful in distinguishing C3 glomerulopathy from other types of glomerular injury. Most renal

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In this issue of JASN, Sethi and colleagues propose that staining for C4d may be useful in distinguishing C3 glomerulopathy from other types of glomerular injury. Most renal


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