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Interleukin-18, neutrophils, and ANCA

BM Pressler, RJ Falk and CA Preston

Hewins *et al.* show that IL-18 is expressed in the kidneys of patients with ANCA-associated glomerulonephritis, and that IL-18 primes neutrophils via p38 MAPK. These findings suggest a role for IL-18, including IL-18-induced T_H1 polarization and IFN-γ production, in the progression of ANCA disease.

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Each cytokine produces a range of cellular responses, dependent on cell type, presence of other modulating cytokines, and other components of the inflammatory milieu. Interleukin-18 (IL-18) was initially named interferon-γ (IFN-γ)-inducing factor, because it was shown to induce production of IFN-γ by splenocytes (particularly in the presence of lipopolysaccharide).[1] We now know that this is due to IL-18's role in polarization of naive T cells to a T helper type 1 (Th1) response, and subsequent production by these T cells of IFN-γ.[2] IL-18 alone is sufficient to induce IFN-γ production by naive T cells, but this effect is significantly amplified in the presence of IL-12 — not surprising, as IL-12 is the most potent Th1-polarizing cytokine described and, in addition, upregulates expression of the IL-18 receptor on T cells.[3] There is still a large amount of handwaving as to why some pathogens induce an IL-12/IL-18/Th1-polarizing response whereas others preferentially lead to an IL-4/Th2-polarizing response, but the prevailing thought is that this depends on the particular set of cytokines induced by a pathogen's binding to a particular subset of pattern-recognition receptors, such as Toll-like receptors. IL-18 may also play a role in some instances of Th2 polarization, particularly in the presence of NK T-cell production of IL-4, although this is not as clear.[2]

IL-18 is constitutively secreted by monocytes, macrophages, and dendritic cells as the inactive precursor pro-IL-18; other cell types have been shown to produce pro-IL-18 as well, but whether this also occurs constitutively is unclear.[2] Pro-IL-18 is activated by enzymatic cleavage by caspase-1, but other proteases can activate IL-18 *in vitro*, including proteinase 3, a component of neutrophil and monocyte azurophilic granules.[4] The IL-18 receptor is a heterodimer that associates after the IL-18R α chain binds activated IL-18. Signal transduction occurs through the IL-18 receptor β chain by several intracellular pathways, including p38 mitogen-activated protein kinase.[2]

Hewins *et al.* report in this issue of *KI* that IL-18 is capable of priming neutrophils *in vitro*, thus augmenting superoxide production by cells after antineutrophil cytoplasmic antibody (ANCA) binding.[5] This increased activity is similar to that seen with tumor necrosis factor-α priming, the favored method for most *ex vivo* experiments studying the effects of ANCA. The authors also demonstrate that neutrophil priming occurs via p38 mitogen-activated protein kinase activation, and that IL-18 and tumor necrosis factor-α may be synergistic at lower concentrations. In addition, increased IL-18 was found in podocytes, distal tubular epithelial cells, and interstitial myofibroblasts and macrophages in renal biopsies from patients with ANCA-associated glomerulonephritis. These findings suggest that an IL-18-induced immune response may play a role in intravascular activation of neutrophils prior to degranulation, or that ANCA production and tissue damage may be maintained by Th1 cells.

But what does this truly mean or imply in ANCA-associated glomerulonephritis? Unfortunately, although production of anti-proteinase 3 or anti-myeloperoxidase immunoglobulin G implies the presence of antigen-specific T cells that have participated in B-cell clonal expansion, affinity maturation, and isotype switching, T cells specific for these granule proteins have not been reliably demonstrated in patients with ANCA disease.[6] There is, however, evidence (albeit often conflicting) that both Th1- and Th2-mediated T-cell responses are involved in ANCA disease. Peripheral blood and intralesional T cells from patients with localized Wegener's granulomatosis are predominantly $CD4^+$ IFN-γ-secreting cells, whereas T cells from patients with Churg-Strauss syndrome are predominantly $CD4^+$ IL-4-secreting cells; Th1 and Th2 cytokine secretion patterns have been reported in T cells from patients with microscopic polyangiitis.[7,8] To confuse matters even more, T cells from patients with ANCA disease may have altered phenotypes (expansion in $CD28^-$ cells) or may alter their cytokine secretion patterns as disease progresses (T cells in generalized Wegener's granulomatosis show a shift to a Th2 pattern).[7,8]

The findings reported by Hewins *et al.*[5] therefore add to our understanding of the Th1/Th2 and cytokine dysregulation in ANCA disease. We can speculate that the finding of IL-18 in renal tissue from patients with ANCA disease, and the ability of this cytokine to prime neutrophils, demonstrate that Th1-mediated responses may be involved in disease progression. However, several additional questions arise from these

[1]*Division of Nephrology, Department of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA*
Correspondence: *RJ Falk, Division of Nephrology, 349 MacNides Hall, University of North Carolina Department of Medicine, Chapel Hill, North Carolina 27599, USA.*
Email: ronald_falk@med.unc.edu

findings. If the podocytes are the primary excreters of IL-18, where are the activated Th1 cells? What is the stimulus for production and secretion of IL-18? Are the podocytes and other renal cells perhaps endocytosing IL-18 rather than producing it? Alternatively, we must ask whether IL-18 production by these cells is instead a consequence rather than an initiator of neutrophil activation. As we know that proteinase 3 is capable of activating pro-IL-18 *in vitro*, what are the functional consequences of this in proteinase 3-ANCA disease? It would be expected that any cytokine secreted by podocytes would pass into the urine rather than cross the glomerular basement membrane opposite to the normal direction of filtration; direct secretion by the podocytes into the vasculature would truly be a novel finding. Regardless, this report reveals that podocytes may be direct participators in the immune dysfunction of ANCA disease, and that IL-18 may be responsible for neutrophil priming *in vivo*.

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Fibroblast growth factor 23: the making of a hormone

SC Schiavi[1]

Fibroblast growth factor 23 (FGF23) modulates serum phosphate and 1α,25-dihydroxyvitamin D3 levels. FGF23 expression is in turn regulated by 1α,25-dihydroxyvitamin D3 and dietary phosphate load, and is strikingly elevated during renal progression. In this issue, Nagano and colleagues report that FGF23 can be modulated by intermittently high dietary phosphate in the presence of compromised renal function.

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Over the past several years, accumulating evidence has documented the role of fibroblast growth factor 23 (FGF23) in the control of phosphate and vitamin D homeostasis. These data show that FGF23 acts in accordance with the definition of a hormone: a circulating protein that targets specific tissues distant from its site of production. FGF23 acts directly on the kidney to regulate the synthesis of 1α,25-dihydroxyvitamin D_3 and the surface expression of the sodium phosphate transporters NaPi-IIa and -IIc.[1–4] Several reports now demonstrate that serum FGF23 concentrations increase both in response to dietary phosphate load with normal renal function and in response to increasing serum phosphate levels associated with progressive renal failure.[1,3] Despite this body of data, a debate continues regarding the physiological relevance of FGF23 as a major regulator of phosphate homeostasis. In particular, it has been argued that elevated FGF23 in renal disease is a consequence of reduced renal clearance and therefore may not play a direct role in the progression of renal disease and its associated complications.

The study presented in this issue by Nagano *et al.*[5] represents an important advancement beyond the previously identified association of elevated FGF23, phosphate, and parathyroid hormone (PTH) levels in renal disease.[1,3,4] By using a model of severe chronic kidney disease and including an experimental arm in which phosphate levels were regulated by intermittent dosing of the phosphate binder sevelamer hydrochloride, the authors have provided convincing evidence that FGF23 serum concentrations were influenced by elevated dietary and serum phosphate. Importantly, the data argue that decreased renal function cannot adequately explain the dramatic rise in FGF23 serum concentrations associated with renal insufficiency, as changes in serum FGF23 levels were observed in the presence of severely compromised kidney function. Together with the substantial data demonstrating direct actions of FGF23 on renal phosphate excretion, these results imply that the levels of FGF23, like those of PTH, may rise in response to the increasing pressure to excrete phosphate.

The study by Nagano *et al.*[5] also demonstrates another important feature of FGF23 regulation: that FGF23 serum levels are modified rather slowly in response to increased dietary phosphate. Although changes in serum phosphate and PTH occurred 1 day after sevelamer treatment or removal, there was an additional lag before FGF23 levels were altered. The mechanism triggering the slow regulation of FGF23 is not clear, but the delay suggests that phosphate does not directly control FGF23 synthesis. This result implies that FGF23's function is most important as a chronic rather than an acute response to changes in phosphate and partially explains the need for

[1]*Endocrine and Renal Sciences, Genzyme Corporation, Framingham, Massachusetts, USA*
Correspondence: *SC Schiavi, Endocrine and Renal Sciences, Genzyme Corporation, 1 Mountain Road, Framingham, Massachusetts 01701-9322, USA. Email: susan.schiavi@genzyme.com*