ANCA signaling: Not just a matter of respiratory burst

Antineutrophil cytoplasmic autoantibodies (ANCA) are in the circulation of nearly 90% of patients with pauci-immune necrotizing glomerulonephritis, microscopic polyangiitis, Wegener’s granulomatosis, and the Churg-Strauss syndrome. Over the last decade, the ANCA scientific community has standardized ANCA testing [1], developed a consensus on the nomenclature of vasculitis [2], and pushed forward the observations that ANCA participate in the pathogenesis of vascular inflammation [3]. Of the number of hypotheses related to how ANCA may cause vasculitis, the most prevalent is based on the work of many investigators who have noted that expression of granule proteins on the surface of neutrophils and monocytes allows for ANCA interaction with surface antigens [4–6]. This interaction results in induction of respiratory burst, degranulation, and release of neutrophil and monocyte products into the microenvironment. These activated leukocytes and granule products then interact with the endothelium resulting in its damage [7] (abstract; Yang et al, ASN 30th Annual Meetings, 1997). In this issue of *Kidney International*, Harper et al provide another step forward in our understanding of how ANCA might contribute to the pathogenesis of vasculitis [8].

The effect of ANCA on neutrophils and monocytes is different from other immunoglobulin (Ig) molecules [4, 9]. Ambiguous and conflicting data have confused the issue as to the extent to which the F(ab\(^9\))\(_2\) portion of ANCA molecule mediates leukocyte activation, what influence F(ab\(^9\))\(_2\): antigen binding has on the Fc receptor engagement, and whether the Fc receptor engagement provides the primary signal for leukocyte activation. A number of technical obstacles inherent in the use of human neutrophils and monocytes explain part of the controversy. Yet, at present, it is reasonable to presume that both parts of the Ig molecule result in a signal for leukocyte activation. In vivo, the intensity and regulation of these signals may provide some of the explanation for spectrum of disease severity. However, the full spectrum of biologic diversity is certainly caused by a number of factors, including differences in antigen expression, host leukocyte activation, the type and amount of circulating chemokines and cytokines, the state of the endothelium, and the nature of T- and B-cell interactions.

Harper et al report that neutrophils from ANCA patients display a higher level of apoptosis and that this increase correlates with higher concentrations of surface proteinase 3 [8]. These findings raise a crucial question of whether these patients express proteinase 3/myeloperoxidase (PR3/MPO) on the cell surface, placing them at risk for the development of ANCA-mediated disease, or whether neutrophils in patients with ANCA are primed in the circulation, thus causing PR3 or MPO plasma membrane expression. A recent report introduced the concept that subsets of neutrophils express PR3 molecules on their surface and that the proportion of neutrophils presenting PR3 is genetically controlled and highly stable [10]. Indeed, the authors found that the phenotype of increased PR3 surface expression was significantly increased in patients with ANCA-associated vasculitis.

Harper et al investigated the effects of ANCA on macrophage signaling pathways through engagement of opsonized neutrophils during phagocytosis [8]. Opsonization of apoptotic polymorphonuclear cells (PMNs) with ANCA enhanced clearance by macrophages and activated the macrophages to produce increased amounts of cytokines. Both phagocytosis and transcription require activation of signaling pathways. In fact, a transmembrane receptor has recently been observed in *C. elegans*, CED-1, that mediates phagocytosis. CED-1 is homologous to mammalian scavenger receptor from endothelial cells (SREC) and bears structural resemblance to growth factor receptors, integrins, and lipoprotein receptors [11].

The molecular mechanisms by which ANCA perturb neutrophils certainly appear to require ANCA binding to antigen. With this view in mind, ANCA F(ab\(^9\))\(_2\): signal input must lend itself to ANCA-specific effects, as implied by data indicating a correlation between higher concentrations of surface PR3 and increased apoptosis [8]. The point of interest in these data relevant to the present discussion lies not so much in the increased apoptosis, but in the implication that F(ab\(^9\))\(_2\): binding engages the signaling components of the apoptotic pathway. Moreover, once the cells become apoptotic, they become unresponsive to ANCA binding and signaling, indicating

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that ANCA require an intact signaling network to mediate a response.

Increased interest in ANCA as a signaling molecule has been fueled by findings that neutrophils respond to the physical cues of ANCA by up-regulating transcription of genes, such as interleukin-1β (IL-1β) and IL-8 [10, 12–15]. Circumstantial evidence has linked ANCA with protein kinase C (PKC) activation and inositol 1,4,5-triphosphate (IP₃) generation [16]. ANCA-induced signaling can synergize with arachidonic acid pathways [17], and with tumor necrosis factor-α (TNF-α) signaling pathways [18]. A major function of signaling networks is to place a value on a signal such that it is either dissipated or converted into further biochemical events. Consequently, in the hierarchical framework of signaling networks, the strongest signal prevails [19]. Propagated signals are split and routed through several different pathways to regulate distinct cellular functions. Theoretically, the signal input produced by ANCA-F(ab')₂ fragments alone would give rise to signals distinct from those of the whole IgG molecule. ANCA signaling is most likely a consolidation of signals produced by both ANCA-F(ab')₂ and ANCA-Fc engagement. These signals are probably not mutually exclusive and the complexity of outputs result in various neutrophil and monocyte functions.

One of the more exciting research challenges today is to determine how ANCA perturb signal transduction pathways to orchestrate specific and unique physiologic responses. New advances in technology, including multifunction micro arrays, may prove useful in examining the broad scope of ANCA-stimulated signaling networks that result in gene activation. Our understanding of the molecular mechanism of ANCA signaling is still in its early days. We have much to learn about the consequences of these signals on the clinical and pathologic phenotype of ANCA vasculitis.

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REFERENCES