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Tie-ing the Antiinflammatory Effect of Angiopoietin-1 to Inhibition of NF-κB

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Activation of the vascular endothelium occurs in many clinical scenarios such as inflammatory or infectious conditions (sepsis), reperfusion injury, and transplant graft rejection.[1–5] Under such circumstances, endothelial activation is primarily induced by cytokines such as tumor necrosis factor (TNF) and interleukin-1β (IL-1β) and vascular permeability factors such as vascular endothelial growth factor (VEGF) that upregulate a number of genes including prothrombotic factors, chemokines, and cell adhesion molecules, many of which are dependent on the action of the pleiotropic transcription factor nuclear factor-κB (NF-κB).[2,6–11] Activated endothelium is compromised in its structural and functional integrity, leading to transmigration of leukocytes into the vessel wall, plasma leakage, and thrombosis.

Angiopoeitin-1 (Ang1) is a vasculogenic factor that induces endothelial cell sprouting, migration, and network formation,[12–15] coordinated processes that are crucial in the development of new blood vessels. Angiopoietins signal via the Tie (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains) family of receptor tyrosine kinases, of which Tie2 is endothelial-cell specific. In addition to its important role in vasculogenesis, Ang1, through Tie2, suppresses activation of endothelial cells as evidenced by its inhibition of vascular cell adhesion molecules and procoagulant tissue factor expression.[16,17] These effects confer on Ang1 potent antiinflammatory properties.[18–20] Although Ang1 affects the expression of many NF-κB–responsive genes, a functional and mechanistic link between Ang1 and NF-κB signaling has not been previously shown.

The report by Hughes et al[21] in this issue of *Circulation Research* provides the first demonstration that the mitigating effect of Ang1 on endothelial cell activation may be because of binding of its activated Tie2 receptor to a protein previously shown to modulate NF-κB activation. Using a yeast two-hybrid screen with the intracellular domain of Tie2 as the bait and a human endothelial cell cDNA library, the authors isolate a cDNA clone that turns out to code for a protein termed ABIN-2 (A20 binding inhibitor of NF-κB activation-2) and show that ABIN-2 also associates with full-length Tie2 in mammalian cells. By demonstrating that ABIN-2-Tie2 association is dependent on phosphorylation of Tie2 (an early event that occurs with binding to Ang1), and that Ang1 stimulates this association in endothelial cells, the authors provide a convincing case for the physiological relevance of this association. Moreover, using a deletion mutant of ABIN-2 that would be expected to interact with Tie2 but does not have the capacity to inhibit NF-κB[22] (and can therefore be presumed to act in a dominant inhibitory fashion with respect to Tie2-ABIN-2 interaction), the authors show that this interaction may have functional relevance in the context of Ang1's effect on phorbol ester–stimulated NF-κB activity. Interestingly, outside of the context of Ang1 inhibition of NF-κB activation, and contrary to prior reports,[22] expression of this mutant augments phorbol ester–stimulated κB activity.

As the name implies, the murine homologue of ABIN-2 was first identified as a protein that interacts with A20, a well-known inhibitor of NF-κB activation.[22] A20, a zinc finger protein, was itself first cloned from endothelial cells as an early-response gene induced by TNF,[23] and whose expression is controlled by NF-κB,[24] thus acting as a negative modulator of NF-κB activation. ABIN-2 functions in a similar capacity by inhibiting NF-κB activation in response to a variety of stimuli (TNF, IL-1, phorbol esters), exacting its effect at a point in the activation cascade identical to that of A20.[22] However, unlike A20, ABIN-2 is constitutively expressed in both murine and human tissues.[21,22] The finding by Hughes et al[21] that ABIN-2 structurally and functionally interacts with Ang1-Tie2 signaling also implicates, although does not prove, a role for A20 in Ang1-induced suppression of stimulated NF-κB activity as well. Speaking against this possibility is the recent report that ABIN-1 (that also binds to A20 through a conserved domain shared with ABIN-2[22]) does not require A20 to suppress NF-κB activity.[25]

Part of the intrigue created by the findings reported by Hughes et al[21] is that ABIN-2 was identified only very recently, and there is a dearth of information about the mechanism(s) by which it inhibits NF-κB. ABIN-2 does not have classic phosphotyrosine-binding domains (such as src homology domain 2 [SH2]) that are necessary for interaction of other proteins (Grb adaptors and p85 subunit of phosphoinositide 3-kinase [PI3K]) to tyrosine-phosphorylated Tie2. The residues on Tie2 and ABIN-2 that are critical for their interaction, and how such an interaction cross-communicates with the NF-κB signaling machinery, to modulate NF-κB activation, remain to be identified, and will surely spur intense investigation in the years to come. Regarding the

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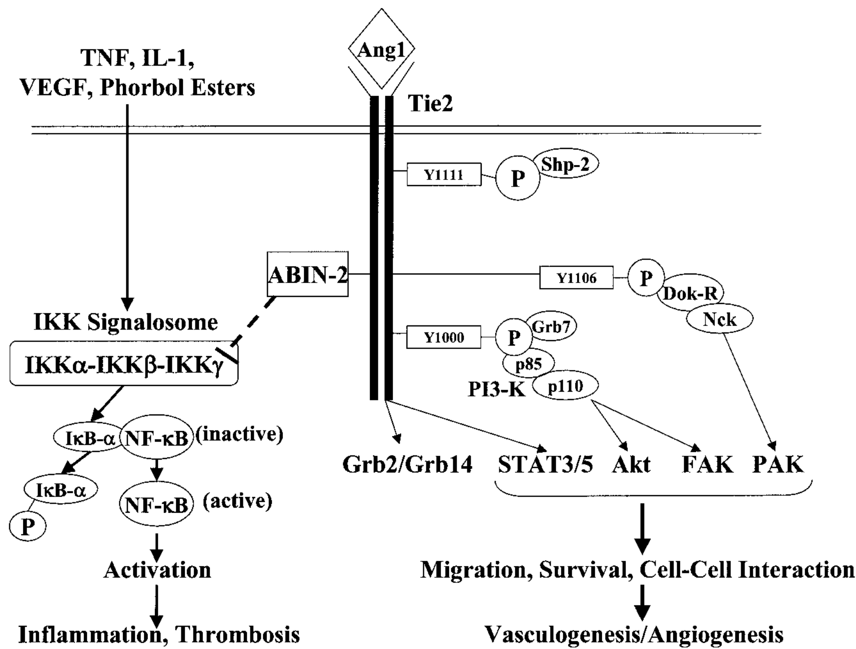

Ang1-Tie2 signaling in endothelial cells. Proposed model showing known (solid line) and potential (dashed line) signaling pathways activated or inhibited by Ang1-Tie2, and proteins known to interact with phosphorylated Tie2, with the specific residues, when known, indicated. The exact nature of, and residues involved in, the Tie2-ABIN-2 interaction is not known. Only one hypothetical mechanism by which ABIN-2 may inhibit NF-κB activation is shown. (Other potential mechanisms, such as via A20, are not shown.) Note: VEGF can also lead to endothelial cell survival, migration, and promote angiogenesis. Ang1 indicates angiopoietin-1; Y, tyrosine residue; Shp-2, protein tyrosine phosphatase; p85, regulatory subunit of PI3K; p110, catalytic subunit of PI3K; Grb, adaptor proteins; STAT, signal transducer and activator of transcription; FAK, focal adhesion kinase; and PAK, p21-activated kinase.

latter, recent provocative evidence suggests that ABIN proteins share a sequence of homology with IKKγ[25] (inhibitor of kappa B kinase), a regulatory component of the IKK signalosome that is essential for phosphorylation and consequent degradation of IκB (inhibitor of kappa B), an indispensable step in NF-κB activation. This raises the interesting possibility that ABIN proteins act by binding to and competing for an upstream regulator of the IKK signalosome, thereby inhibiting its activity. One could then speculate that Ang1-stimulated recruitment of ABIN-2 in endothelial cells could facilitate this process (perhaps by leading to a conformational change in ABIN-2 or bring it in proximity to proteins involved in IKK activation) (Figure).

In summary, the data offered by Hughes et al[21] show that the Tie2-ABIN-2 interaction is responsible for the inhibitory effects of Ang1 on stimulated NF-κB activity, implicating this novel interaction in mediating the antiinflammatory properties of Ang1. However, in addition to its role in inflammation, NF-κB has myriad effects, raising numerous questions as to how, if at all, the Tie2-ABIN-2 interaction modulates the other effects of Ang1 on endothelial cells (Figure). For example, what role, if any, does ABIN-2 play in Ang1-stimulated endothelial cell survival[26] and migration,[15] both of which are critical in angiogenesis, and both of which in endothelial cells or other cellular systems involve NF-κB signaling in some fashion.[27–29] Similar questions arise about the role of the Tie2-ABIN-2 interaction in molecular crosstalk with signaling pathways, such as PI3K-Akt, known to be activated by Ang1 (Figure), but that also modulate NF-κB activity.[30] The answers to such questions will be crucial in determining the physiological relevance of the Tie2-ABIN-2 interaction and may provide important clues to the intricate process of blood vessel formation in normal physiology and disease states.

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