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NOVEL EFFECTS OF INFLAMMATORY CELL PROTEASES ON VASCULAR ENDOTHELIA

Preston GA, Pendergraft WF, Yang JJ, Jennette JC, and Falk RJ. University of North Carolina at Chapel Hill, Chapel Hill, NC, USA 27599.

Leukocytes secrete enzymes upon stimulation, which are key effectors of vascular inflammation. In particular, the T-cell protease granzyme B can directly activate target cell caspases responsible for apoptotic execution. The neutrophilic pseudoprotease azurocidin has been linked with increased vascular permeability and edema. The studies herein reveal novel functions of two additional neutrophilic proteases, proteinase 3 and elastase, extending their role in inflammatory disease beyond the described functions of extracellular matrix degradation. Because leukocytes release millimolar amounts of proteases at sites of inflammation, we investigated the effects of neutrophil serine proteases, proteinase 3 and elastase, on the function and survival of human umbilical vein endothelial cells (HUVEC).

We report that endothelial cells internalize proteolytically active proteinase 3 into endosomal-like vesicles. Once internalized, PR3 cleaves NF-κB (p65) in the N-terminal region, generating a fragment of ~56kDa that is dysfunctional as a transcription factor. Protein sequence analysis of the N-terminal amino acids of the PR3 generated fragment showed cleavage at the $VGKDC^{95}$ -R^{96} motif of p65, two amino acids upstream of the reported caspase 3 site. We found that elastase also inactivates NF-κB function through direct cleavage in the C-terminal domain. Caspase 3 inhibitors did not block this cleavage. Treatment with proteinase 3 or elastase results in apoptosis. However, NF-κB cleavage alone is not sufficient to induce death pathways. To determine the signaling pathways utilized by proteinase 3 and elastase in the activation of apoptosis, signaling molecules of known stress pathways were examined using antibodies specific for the active forms. Our data indicate that both p38 MAPK and JNK pathways are responsive to protease treatment. Inhibition of JNK or p38 with the inhibitor SB203580 reduced proteinase 3-induced apoptosis by ~75%.

Direct cleavage of NF-κB by proteinase 3 and elastase as reported here, combined with reports of cleavage of IL-1β and Sp1, indicates that these proteases possess caspase-like functions. Proteinase 3 and elastase are noncaspase proteases secreted by neutrophils at sites of acute inflammation that have the capacity to mediate intercellular caspase-like functions.