Neutrophil elastase induces IL-8 synthesis by lung epithelial cells via the mitogen-activated protein kinase pathway.

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Abstract

The sequestration of neutrophils in the lung and the release of proinflammatory mediators, including neutrophil elastase, are responsible for sepsis-induced microvascular permeability and alveolar epithelial cell damage. To assess the underlying mechanism, human neutrophil elastase (0.01-0.5 microg/ml) was added to cultured A549 epithelial cells in the presence or absence of inhibitors. IL-8 was analyzed by ELISA or by RT-PCR to measure the IL-8 synthesis capacity. Mitogen-activated protein kinase (MAPK) activity was detected by Western blot analysis. Neutrophil elastase dose-dependently increased IL-8 release from cultured A549 epithelial cells. Pretreatment with a specific elastase inhibitor, elastase inhibitor II (at 0.5, 5, and 50 microg/ml), dose-dependently inhibited neutrophil elastase-induced IL-8 release. The activities of MAPK, p38, and extracellular signal-regulated kinase (ERK) were upregulated by neutrophil elastase. Nuclear transcriptional factor-kappa B (NF-kappaB) and activator protein 1 (AP-1) were also activated. These responses were significantly inhibited by elastase inhibitor II. A specific inhibitor of p38 MAPK (SB203580) and an NF-kappaB inhibitor (pyrrolidine dithiocarbamate), but not an ERK inhibitor (PD 98059), significantly inhibited neutrophil elastase-induced IL-8 release and mRNA expression. The specific tyrosine kinase inhibitor, genistein, and the protein kinase C (PKC) inhibitor, Ro 31-8220, also inhibited IL-8 release and mRNA expression as well as p38 and NF-kappaB activation. There was no significant effect by the protein kinase A inhibitor, H-89, on neutrophil elastase-induced IL-8 synthesis or p38 MAPK activation. Our results indicate that neutrophil elastase activates p38 MAPK which upregulates NF-kappaB and AP-1 activities, thus inducing IL-8 mRNA expression and protein synthesis. Tyrosine kinase and PKC are implicated in neutrophil elastase activation of the MAPK pathway.