

Neutrophil elastase affects not only tissue damage, but it also regulates hemotopoiesis.

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Neutrophil elastase (NE) is an abundant serine proteinase stored in the azurophilic granules of neutrophils along with cathepsin G (CathG) and proteinase-3 (PR3), and elevated serum levels or overexpression of these proteinases has been reported in cases of severe inflammations such as sepsis, autoimmune diseases, etc [1, 2]. NE is released to the circulation upon activation of neutrophil by inflammatory factors such as lipopolysaccharide (LPS) and inflammatory cytokines, tumor necrosis factor and interleukin-1, 6 and 8, etc. These proteinases are considered to work for host defense by killing bacteria associated with infectious diseases [1, 3]. However, the excess release of NE from activated neutrophil in severe sepsis mediates tissue damage through the proteolytic cleavage of cell surface glycoproteins, extracellular matrix and junctional complexes [4, 5] as well as soluble proteins. An excessive release of NE may thus cause single or multiple organ failure due to the above tissue damages.

NE also cleaves fibrin-related products [6] and metalloproteinase with thrombospondin type 1 motif, 13 (ADAMTS13) [7]. Therefore, excess NE may cause a fibrinogenolysis, fibrinolysis and hypofibrinogenemia in patients with disseminated intravascular coagulation (DIC) [8] due to acute

promyelocytic leukemia (APL) or severe sepsis. An increase in the amount of fibrin degradation products by granulocyte derived elastase (GE-XDP) could be useful for the diagnosis of DIC or activation of neutrophil. The reduction of ADAMTS13 increases the ultra large multimers of von Willebrand factor, thus resulting in the activation of platelets, and leading to the formation of disseminated micro-thrombi. The NE in the systemic circulation is inhibited by  $\alpha$  1-antitrypsin released from the liver and hematopoietic cells. As large amount of  $\alpha$  1-antitrypsin is present in the blood, whereas NE usually exerts its effects locally near the activated neutrophils. Hereditary  $\alpha$  1-antitrypsin deficiency is associated with pulmonary emphysema. NE was considered to be not only a factor providing a defense against microorganisms but also as a factor exacerbating inflammation, DIC and organ failure. In DIC, elevated fibrin-related markers, hypofibrinogenemia, thrombocytopenia, increased or decreased neutrophil counts, anemia or organ failure are sometimes observed.

Recently, the effects of NE on hematopoiesis have been recognized, but the detailed mechanism underlying its regulation of this process is not well

understood. NE might affect the hematopoietic cells including differentiated cells and hematopoietic progenitor cells (HPCs), through direct enzyme activity or induction of apoptosis. Neutropenia due to sepsis is caused by not only direct destruction of neutrophils by several factors including NE but also to feedback in response to suppressed granulopoiesis through direct proteolytic action on granulocyte-colony stimulating factor (G-CSF) [9]. The serine proteinases such as NE, CathG and PR3 which are synthesized and released from hematopoietic cells, affect the in vitro proliferation of HPCs. These proteinases rapidly and dose-dependently degrade hematopoietic growth factors (HGFs) in medium, thus resulting in the decreased proliferation of the HPCs [10].

Mutations in the genes related to the regulatory system for NE synthesis are associated with hereditary neutropenia, including severe congenital neutropenia (SCN) and cyclic neutropenia [11]. SCN is a heterogeneous disorder of myelopoiesis which follows an autosomal dominant or autosomal recessive pattern of inheritance. Mutations in ELA2 encoding the neutrophil granule protease, NE, are the major cause of cyclic neutropenia and SCN. Genetic analyses in SCN patients have indicated the

presence of mutations in the ELA2 gene in most patients. Skokowa J et al [12] demonstrated that the ELA2 mRNA expression in myeloid progenitor cells and the plasma protein levels of NE were markedly reduced in patients with SCN harboring mutations in either ELA2 or HAX-1 genes. The ELA2 gene promoter is positively regulated by the direct binding of LEF-1 or C/EBPalpha. The transduction of hematopoietic cells with LEF-1 cDNA resulted in the up-regulation of ELA2/NE synthesis. LEF-1 rescues CD34(+) cells isolated from patients with SCN, and resulted in the granulocytic differentiation of the cells which was in line with increased levels of functionally active ELA2/NE present in these cells. A canine form of cyclic neutropenia corresponds to human Hermansky-Pudlak syndrome type 2 and results from mutations in the AP3B1 gene encoding a subunit of a complex involved in the subcellular trafficking of vesicular cargo proteins.

In addition, NE can induce the apoptosis of hematopoietic progenitor cells, which is prevented by a secretory proteinase inhibitor [11]. Dokai et al [13] demonstrated that hematological cells might be affected by NE, which is regulated by endogenous  $\alpha$ 1-antitrypsin under the stimulation of

LPS, suggesting that granulocytes could protect themselves from NE-induced cellular damage by efficiently neutralizing its activity with concomitant secretion of endogenous  $\alpha$ 1-antitrypsin. Supporting this suggestion, neither K562 nor MEG-01 cells, which do not release  $\alpha$ 1-antitrypsin, could protect themselves from NE-induced cellular damage. Although these experiments were done under serum-free conditions, a large amount of  $\alpha$ 1-antitrypsin exists in the serum. The K562 cells are of erythrocyte lineage, and this model suggests that NE may be related to the anemia in sepsis and chronic inflammation. MEG-01 cells are of megakaryocyte lineage, and this model indicates that NE is involved in at least one of the mechanisms underlying thrombocytopenia in chronic inflammation.

Some cell signaling by NE may cause apoptosis. Although the cell surface receptor involved in NE-induced apoptosis has not been clearly identified, several candidates have been suggested, such as protease-activated receptor (PAR)-1, PAR-2, and Toll-like receptor (TLR)-4. NE, thrombin, and PAR-1-activating peptide (PAR-1AP), but not the control peptide induced apoptosis in human airway and alveolar epithelial cells

[14]. These effects were largely prevented by a specific PAR-1 antagonist and by short interfering RNA directed against PAR-1. These data suggest that NE mediates the apoptosis of human lung epithelial cells through PAR-1-dependent modulation of the intrinsic apoptotic pathway via alterations in mitochondrial permeability and by modulation of JNK and Akt [14]. NE-induced apoptosis of lung epithelial cells is also mediated by a PAR-1-triggered pathway involving the activation of NF-kappaB and p53, and a PUMA- and Bax-dependent increase in mitochondrial permeability leading to the activation of distal caspases. Further, p53 contributes to NE-induced apoptosis by both transcriptional and post-transcriptional mechanisms [15]. TLR-4 is expressed in K562, MEG-01 and HL-60 cells [16] and NE might be associated with expression of the TLR-4 on monocytes and macrophages in the septic state [17]. Therefore, TLR-4 is considered to be the most likely candidate for cell surface receptor for NE.

Finally, NE is well recognized as an most important mediator in sepsis and inflammation, and it might affect the hematopoiesis. The mechanism responsible for the regulation of hemotopoiesis by NE should be examined in greater detail in future studies.

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