Apopitosis (Programmed Cell Death) and Functional Changes in Aging Neutrophils*

Modulation by Inflammatory Mediators


Despite the injurious potential of neutrophil (PMN) contents, their fate at inflamed sites is poorly understood. Although it is widely assumed that PMNs inevitably disintegrate before their removal by local macrophages, an alternative fate has been described by which aging PMNs, derived from blood or from inflamed sites, undergo apoptosis, which leads to macrophage ingestion of the intact senescent PMN. Macroage recognition of apoptotic PMNs occurs via a novel mechanism that does not appear to stimulate the macrophage to release proinflammatory mediators. The functional properties of aging PMNs and the effect of inflammatory mediators on the rate of PMN apoptosis would also have important implications for the control of inflammation.

A number of important PMN functions declined in concert with the appearance of apoptotic cells in pure populations of PMNs aging in vitro. These functions included chemotaxis, phagocytosis of opsonized zymosan, stimulated shape change, and stimulated release of granule enzymes, but there was no evidence of loss of membrane integrity as assessed by trypan blue exclusion. When aged PMNs were separated by counterflow centrifugation into apoptotic and nonapoptotic subpopulations, functional loss appeared to be restricted to the apoptotic fraction, which suggested that apoptosis may also serve as an "inert" packaging mechanism for neutrophil contents prior to macroage removal. When inflammatory agents were included with PMNs aging in vitro, bacterial lipopolysaccharide (LPS), granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemotactic peptide C5a and its synthetic counterpart FMLP were all found to inhibit, in a concentration-dependent fashion, the rate of PMN apoptosis in vitro without causing PMN aggregation or loss of membrane integrity (by trypan blue exclusion). For example, after 13 h in culture, untreated PMN populations were 43.3% ± 4.8% apoptotic (mean ± SE; n = 7) whereas cell populations treated with LPS at 100 ng/ml were 25.9% ± 4.5% apoptotic, at 1 μg/ml were 12.9% ± 2.9% apoptotic, and at 10 μg/ml were 6.1% ± 0.9% apoptotic. Similar reductions in the rate of apoptosis was seen following treatment with GM-CSF, C5a, and FMLP, which suggested that the process of apoptosis can be modulated and PMN longevity can be prolonged by factors that are present in high concentration at sites of inflammation.

We hypothesize that PMN apoptosis is a modulatable process that, by "packaging" PMN contents, leads to macrophage removal of the intact cell without the release of further proinflammatory macrophage products, thus representing an injury-limiting PMN disposal mechanism important in inflammatory resolution.

REFERENCES
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Macrophage Vitronectin Receptor, CD36, and Thrombospondin Cooperate in Recognition of Neutrophils Undergoing Programmed Cell Death*

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Macrophage phagocytosis of intact senescent neutrophils (PMNs) is an important mechanism of PMN clearance from the resolving inflamed site which is determined by programmed cell death or apoptosis in the aging PMN. This leads to recognition of the aged PMN as "senescent self" and phagocytosis by macrophages via a mechanism in which the Arg-Gly-Asp adhesion signal and a macrophage β3 integrin, the vitronectin receptor (VnR), play critical roles.

However, the possible involvement of additional cell-surface signaling mechanisms was suggested by specific inhibition of macrophage phagocytosis of aged PMNs by amino sugars and basic amino acids, molecules known to inhibit thrombospondin (TSP)-dependent adhesive properties of activated platelets. Since macrophages both secrete TSP and bear receptors for TSP, which include CD36, we sought evidence of TSP/CD36-mediated intercellular adhesion in macrophage recognition of aged PMNs.

Attachment of macrophages to surfaces coated with TSP specifically inhibited subsequent recognition of aged PMNs by over 80%, indicating that TSP-binding structures on the macrophage (downregulated by this maneuver) played a major role in the recognition mechanism. Indeed, there was evidence that TSP acts as a "molecular bridge" between the aged PMN and the macrophage: (1) TSP in solution at 5 μg/ml enhanced aged PMN uptake, whether in solution in the

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