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REVIEW

Stanford L. Peng

Neutrophil apoptosis in autoimmunity

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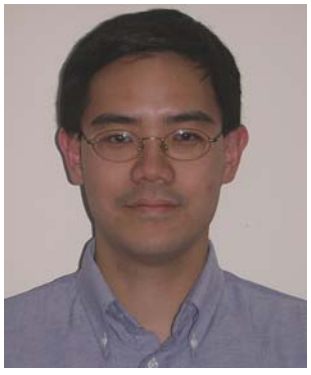
Abstract The regulation of death pathways in neutrophils has been of long interest, but the relevance of neutrophil apoptosis to the pathogenesis and treatment of autoimmune diseases has only recently been becoming recognized. This brief review addresses the relevant pathways by which neutrophil apoptosis is regulated and summarizes the current understanding regarding the potential role of apoptotic neutrophils in the initiation and/or propagation of autoimmunity, as well as the applicability of modulation of neutrophil death in the treatment of autoimmune diseases.

Keywords Neutrophils · Arthritis · Lupus · Inflammatory bowel · Innate immunity · Apoptosis



Stanford L. Peng received his M.D. and Ph.D. in biology from Yale University, followed by internal medicine and rheumatology training at the Hospital of the University of Pennsylvania and Brigham and Women's Hospital, respectively. He is presently Director of Arthritis Research in the Inflammation, Autoimmunity and Transplantation Research department at Roche Palo Alto, California, USA. His research interests include molecular mechanisms of autoimmunity and tolerance.

An overview of neutrophil apoptosis

Neutrophils play critical effector and regulatory roles in a multitude of immune responses, eliminating undesired pathogens and other components of their environment by phagocytosis, the elaboration of toxic mediators, and the production of effector cytokines, which in turn recruit and modulate the activity of other cells [1]. Their activity appears to be tightly regulated, at least in part, by apoptosis, which results in a half-life of approximately 8–10 h or less in both inflammatory and noninflammatory states [2, 3]. Apoptosis appears to provide an appropriate means by which to eliminate unwanted or unnecessary neutrophils: this process eliminates cells from inflamed tissues without releasing their hazardous contents, and the uptake of apoptotic cells by surrounding phagocytic cells is associated with the release of anti-inflammatory mediators. In this way, neutrophil death via apoptosis likely makes a critical contribution to the regulation of inflammatory responses, in both pathological and nonpathological, physiological states [1].

Neutrophil apoptosis reflects a balance between both death and survival factors, some of which appear to be cell lineage specific (Fig. 1). Spontaneous neutrophil apoptosis may reflect clustering of death receptors (DRs), such as Fas (CD95), perhaps in response to reactive oxygen species (ROS) [4], leading to caspase signaling and/or mitochondrial membrane depolarization. However, this process is likely influenced by other pathways as well, particularly pro- and antiapoptotic proteins of the bcl-2 family, as well as survival factors like granulocyte-macrophage colony-stimulating factor (GM-CSF), which elicit signaling at least via phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways [5]. Other modulators of apoptosis and survival include the tyrosine phosphatase Src homology domain 2 (SH2)-containing tyrosine phosphatase-1 (SHP-1), which inactivates at least some Src family kinases [6], and the cystein protease calpain, which modulates Bax and caspase-3 [7], each of which is known to regulate apoptosis in primary neutrophils. Other biological pathways likely contribute as well, such as type I interferons via the phosphoinositol 3-kinase,

S. L. Peng (✉)
Inflammation, Autoimmunity, and Transplantation Research,
Roche Palo Alto,
3431 Hillview Avenue, M/S R7-101,
Palo Alto, CA 94304, USA
e-mail: stanford.peng@roche.com
Tel.: +1-650-8555649
Fax: +1-650-8555501

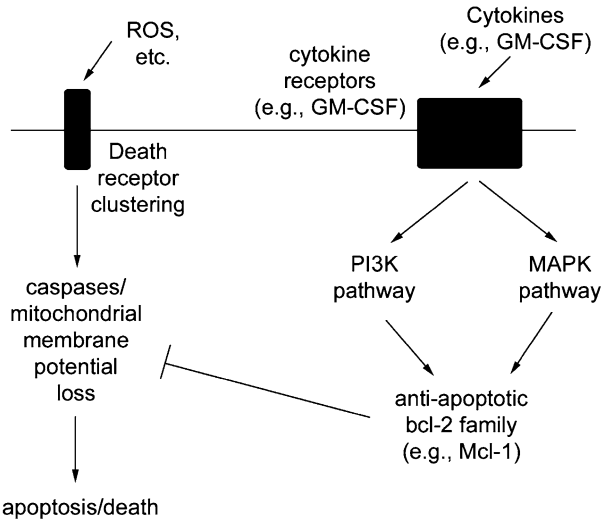


Fig. 1 A simplified view of the regulation of neutrophil death and survival. In general, neutrophil survival reflects a balance between death and survival signals. Spontaneous neutrophil apoptosis may reflect, at least in part, clustering of death receptors (DRs), such as Fas (CD95), perhaps in response to reactive oxygen species (ROS), leading to caspase signaling and/or mitochondrial membrane depolarization. This process is modulated by other pathways, especially survival factors such as cytokines (e.g., granulocyte-macrophage colony-stimulating factor, GM-CSF), which elicit signaling at least via the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways, promoting survival via antiapoptotic bcl-2 family members and the inhibition of proapoptotic caspase activities

protein kinase C-δ, and NF-κB pathways [8], or immunoglobulins and/or their immune complexes via Fc receptors such as FcγRIII [9]. Thus, a growing list of intracellular modulators regulate neutrophil apoptosis, the specific hierarchy and organization of which remain incompletely elucidated. For a more detailed discussion of neutrophil apoptosis, the reader is directed to one of several recent reviews (e.g., [10]).

Modulation of neutrophil apoptosis as a pathogenic mechanism in autoimmunity

Perhaps the most straightforward mechanism by which modulation of apoptosis might promote inflammatory disease involves defective neutrophil apoptosis, which could allow improper neutrophil survival and thereby promote inflammation and tissue damage. Several diseases have been associated with diminished neutrophil apoptosis and increased effector neutrophil numbers: in crystal-induced arthritis, for example, this may result, at least in part, from direct antiapoptotic effects of monosodium urate or calcium pyrophosphate crystals [11, 12]. In rheumatoid arthritis, this may reflect activities in inflamed synovial fluid, perhaps adenosine [13], while in inflammatory bowel disease, this may reflect increased local concentrations mucosal G-CSF [14]. Thus, survival or other antiapoptotic factors associated with some diseased organ(s) can promote neutrophilia and inflammation (Table 1).

However, neutrophils in many autoimmune diseases are associated with an increased propensity towards apoptosis. In rheumatoid arthritis, for instance, inflamed synoival fluid often contains oligonucleosomal material consistent with apoptotic neutrophils [15]. Systemic lupus erythematosus (SLE) is characterized by an overrepresentation of apoptotic neutrophils, which may reflect a disease-specific defect in the clearance of apoptotic cells [16], the ability of autoantibodies like anti-DNA to bind to and/or penetrate cells and promote apoptosis [17], and/or abnormally elevated levels of proapoptotic factors like tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL [18]). Similarly, increased neutrophil apoptosis has been observed in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides, where apoptotic neutrophils may in fact provide a source of immunologically exposed neutrophil antigens that promote the production of ANCAs [19–21]. Indeed, some studies have indicated that ANCAs preferably bind antigens in apoptotic, vs viable, neutrophils [22], either way modulating their apoptotic pathways [23]. Similar effects on neutrophils have been reported for other autoantibodies, such as anti-La in SLE [24]. In this way,

Table 1 Autoimmune/rheumatic diseases associated with abnormalities in neutrophil apoptosis

Disease	Proposed pathogenic mechanism(s)
Diseases associated with diminished neutrophil apoptosis	
Crystalline arthritis	Inhibition of apoptosis by MSU or CPP crystals
Inflammatory bowel disease	Elevated G-CSF in locally inflamed mucosa
Rheumatoid arthritis	Inhibition of apoptosis by synovial fluid adenosine
Diseases associated with increased neutrophil apoptosis	
ANCA-related vasculitis	Proapoptotic effects of autoantibodies on neutrophils
	Autoimmunogenic effect of apoptotic neutrophils on ANCAs
Systemic lupus erythematosus	Defective clearance of apoptotic cells
	Proapoptotic effects of autoantibodies on neutrophils
	Elevated circulating levels of proapoptotic factors, e.g., TRAIL

Tabulated are rheumatic/autoimmune diseases in which neutrophil apoptosis has been specifically reported to be abnormal
ANCA Antineutrophil cytoplasmic antibody, *CPP* calcium pyrophosphate, *G-CSF* granulocyte colony-stimulating factor, *MSU* monosodium urate, *TRAIL* tumor necrosis factor (TNF)-related apoptosis-inducing ligand

neutrophil apoptosis can partake in a self-propagating cycle in autoimmunity, promoting the generation of pathogenic inflammatory mediators, such as autoantibodies, which in turn further augment neutrophil death (Table 1).

Modulation of neutrophil apoptosis as a therapeutic strategy

Modulation of neutrophil death may therefore facilitate two potential outcomes: on one hand, in diseases like ANCA vasculitis, therapeutic inhibition of apoptosis may actually help suppress the supply of apoptotic material which promotes pathogenic inflammatory mediators like autoantibodies. However, on the other hand, neutrophilic inflammation clearly remains a critical component of the inflammatory lesions in many autoimmune diseases, including arthritis, lupus, and vasculitis, such that the therapeutic induction of neutrophil death continues to appear the more therapeutically viable approach. However, few studies, if any, have addressed the relative impact of such approaches in specific disease indications; thus, current assessments must rely on studies which correlate the therapeutic effect of an intervention upon neutrophil survival.

A few widely used therapies in autoimmune diseases are though to act, at least in part, by modulating neutrophil apoptosis: sulfasalazine, for instance, promotes apoptosis in neutrophils but not in other leukocytes [25], and auranofin (gold) inhibits neutrophil apoptosis at low concentrations but induces neutrophil death at high concentrations [26]. Intravenous immunoglobulin (IVIg) preparations generally contain proapoptotic anti-Fas antibodies, which promote neutrophil apoptosis [27, 28]. Such interventions clearly also exert effects outside of apoptosis per se—e.g., the ability of IVIg to interact with Fc receptors—but their concomitant ability to affect neutrophil survival suggests that at least part of their therapeutic benefit derives from an effect on neutrophil functions.

In addition, some animal models have indirectly demonstrated the potential utility of neutrophil apoptosis modulation in autoimmune disease. For instance, in a peroxochromate-induced mouse model of arthritis, nicotinamide—an inhibitor of poly(ADP-ribose) polymerase (PARP), a nuclear enzyme which participates in the apoptotic response to oxygen- and nitrogen-radical-induced DNA strand breaks—suppressed disease severity [29]. In addition, 3-aminobenzamide and 1,5-dihydroxyisoquinoline—also PARP inhibitors—successfully ameliorated trinitrobenzene sulfonic acid-induced colitis in rats [30]. Mice deficient in the forkhead transcription factor Foxo3a were refractory to the K/BxN serum transfer model of arthritis due to an inability of their neutrophils to suppress Fas ligand-induced apoptosis [31]. Such findings interestingly suggest that, whereas the life span of inactive neutrophils might be regulated by Fas-induced apoptosis [4], inflammatory neutrophils generally may suppress apoptosis via Fas, such that alternate pathways (e.g., bcl-2 family members) regulate neutrophil life span in disease states [31]. In fact, apoptosis-inducing anti-Fas antibodies can effectively treat arthritis in several mouse models, including collagen-induced arthritis, MRL/gld mice, and human T-cell leukemia virus (HTLV)-1 Tax transgenic mice, and disease in HTLV-1 env-pX transgenic mice is exacerbated on an *lpr* (Fas-mutant), yet improved on a Fas-transgenic, background [32–35]. On the other hand, however, DBA/lpr mice are less susceptible to collagen-induced arthritis than their DBA/+ counterparts [36]. In all these studies, it remains unclear how much of the phenotypes reflect the specific role of Fas on neutrophils, as opposed to other cell lineages susceptible to Fas-induced apoptosis, such as T cells or synovial cells; still, the contextual importance of the Fas pathway in autoimmune-related neutrophil survival clearly remains incompletely elucidated. Nonetheless, although indirect, such accumulating evidence suggests that interventions which ameliorate autoimmune diseases like arthritis may work, at least in part, by affecting neutrophil survival and/or apoptosis.

Conclusions

The relevance of neutrophil apoptosis and death to the pathogenesis and therapy for autoimmune diseases has only relatively recently begun to become recognized. In some diseases, such as lupus or ANCA-associated vasculitis, apoptotic neutrophils may both promote and result from ongoing autoimmune processes, but in all or nearly all autoimmune diseases, neutrophils can also be considered critical effectors of inflammation. Thus, the induction of neutrophil death remains a viable desired therapeutic outcome, but the relative values of inhibiting vs promoting neutrophil death in specific disease states remain to be investigated and understood. Needed are studies which directly address the processes that regulate neutrophil death in both physiological and pathological states.

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