Review

Living and dying for inflammation: neutrophils, eosinophils, basophils

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Neutrophils, eosinophils, and basophils play essential roles during microbe-induced and sterile inflammation. The severity of such inflammatory processes is controlled, at least in part, by factors that regulate cell death and survival of granulocytes. In recent years, major progress has been made in understanding the molecular mechanisms of granulocyte cell death and in identifying novel damage- and pathogen-associated molecular patterns as well as regulatory cytokines impacting granulocyte viability. Furthermore, an increased interest in innate immunity has boosted our overall understanding of granulocyte biology. In this review, we describe and compare factors and mechanisms regulating neutrophil, eosinophil, and basophil lifespan. Because dysregulation of death pathways in granulocytes can contribute to inflammation-associated immunopathology, targeting granulocyte lifespan could be therapeutically promising.

Granulocytes in inflammation

Granulocytes (neutrophils, eosinophils, and basophils) play important roles in inflammation: both for clearing pathogens and for immunoregulation (Box 1). They mature in the bone marrow, are released fully differentiated into the circulation, and remain in the resting G_0 phase of growth for the duration of their rather short life [1–3]. In the absence of any extracellular stimuli, circulating neutrophils undergo spontaneous apoptosis within 1–5 days; estimates vary depending on the analytical method employed [1]. The removal of apoptotic neutrophils occurs via uptake by phagocytes in the liver, spleen, and bone marrow [4], and a coordinated action between apoptotic neutrophil removal and granulopoiesis within the bone marrow has been demonstrated [5]. By contrast, eosinophils marginate to a large extent from the circulation to tissues, where they reside for 2–5 days [3]. The fate of dying eosinophils and basophils under noninflammatory conditions *in vivo* has not been reported.

Granulocytes migrate from the blood into tissues in response to chemoattractants, such as interleukin (IL)-8 (neutrophils) or eotaxin (eosinophils and basophils). Upon extravasation and stimulation by cytokines and/or molecules derived from pathogens or damaged cells granulocytes become fully active. At the inflamed site, they use a diverse array of toxic effector molecules to combat invading microorganisms. Although these secretory mechanisms are aimed at the invading pathogens, they are certainly cytotoxic towards host tissue cells too. Therefore, a finely tuned balance of antimicrobial defence versus host damage is of the utmost importance. In addition to fighting pathogens directly, granulocytes also exhibit important immunomodulatory properties and have been shown to produce a plethora of cytokines, chemokines, and other proinflammatory mediators [6–8].

The course of inflammation is partly determined by the lifespan of inflammatory granulocytes within tissue, which is increased in inflammatory neutrophils as compared with circulating neutrophils [9] and may reach approximately 2 weeks for eosinophils, at least under *ex vivo* conditions [10]. Delaying apoptosis is an important mechanism for granulocyte accumulation at sites of inflammation, but may also prolong the inflammatory response [11]. Recent studies have identified new factors that regulate granulocyte survival, including several novel cytokines [12], damage-associated molecular pattern molecules (DAMPs), other activities in the microenvironment [13–15], and also pathogen constituents [16]. By contrast, limiting granulocyte survival via ligation of death receptors [17], phagocytosis of pathogens [16], or reduced production of survival factors during the resolution phase [12] is critical for controlling a granulocytic inflammatory response. Importantly, delayed granulocyte apoptosis has been shown in association with several inflammatory diseases (Table 1).

A number of studies have uncovered important differences between human and mouse granulocytes. For instance, human neutrophils differ greatly in blood counts from their mouse counterparts and show a diverging antimicrobial repertoire, for example defensin expression [18] and serine protease specificity [19], as well as varied intracellular signalling pathways, for example phosphatidylinositol 3 kinase (PI3K) subunit activation [20]. Eosinophils and neutrophils differ in cell surface pattern (e.g., IgA receptor expression) between mice and humans and, consequently, responsiveness to certain stimuli n-Formyl-Met-Leu-Phe (fMLF) receptor activation in eosinophils). Moreover, cytokine and/or mediator production, for example IL-10 generation [21], and reactive oxygen species (ROS) generation upon fungal infection [22], differ between human and mouse neutrophils. Although mouse *in vivo* inflammatory models are invaluable tools to gain insight into the potential roles of granulocytes during inflammatory

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Box 1. Biological functions of neutrophils, eosinophils, and basophils

Granulocytes are characterised by granules that can be released from the cytosol into the extracellular space upon stimulation. Although neutrophils and eosinophils use the molecules stored in these granules to kill microorganisms, the granules of eosinophils and basophils are rich in soluble mediators, such as cytokines, that, among others things, orchestrate hypersensitivity reactions. In addition, each cell type uses specific cellular components to modulate the immune response as outlined below.

Until recently, granulocytes were mainly perceived as those cells that constitute the first line of defence against invading microorganisms. Whereas neutrophils remove smaller pathogens like bacteria and viruses by phagocytosis and intracellular killing, every kind of pathogen, including larger and more-complex intruders, can be attacked by granulocytes via the release of different mediators from their granules. These granule components include proteolytic enzymes (cathepsin G, proteinase-3, elastase), cytotoxins (lysozyme, major basic protein, eosinophilic cationic protein, peroxidases), antimicrobial peptides (defensins, LL-37), and molecules belonging to the humoural arm of innate immunity (pentraxin-3, ficolin-1) [94]. Moreover, neutrophils and eosinophils produce reactive oxygen species (ROS) required for the formation of DNA-containing extracellular traps, which bind and kill microorganisms [87,88].

During the past decade, our understanding of the role of granulocytes broadened substantially as the repertoire of known granulocyte function has expanded. Hence, neutrophils have been shown to produce a large variety of proinflammatory cytokines as well as pro-resolving mediators [6]. In addition, the direct interaction of neutrophils with macrophages, dendritic cells, natural killer cells, and lymphocytes modulates the immune response [6]. Thus, neutrophils may not only orchestrate the onset of inflammation but also its resolution.

In addition to destroying larger microorganisms by the release of cytotoxins, eosinophils can modulate innate and adaptive immunity by producing immunoregulatory cytokines, such as interleukin (IL)-4, IL-8, IL-10, and IL-13. Furthermore, they are not only drivers of allergy but also contribute to tissue repair and remodelling [7]. Basophils are known to produce large amounts of IL-4, IL-13, and histamine, thus modulating allergic responses [8].

responses, differences observed between mouse and human granulocytes clearly generate difficulties in translation of mouse preclinical studies into human therapies.

In this review, we refer, unless otherwise indicated, to data obtained from human studies. Specifically, we will discuss similarities and differences between factors modulating the lifespan of neutrophils, eosinophils, and basophils in inflammation. Moreover, we will focus on recent studies that identify new molecular players involved in granulocyte death and survival or that help to unravel the role of cell–cell interactions in this context. A summary of the survival and death factors regulating the lifespan of granulocytes can be found in Tables 2 and 3. The potential sources of these factors are shown in Figure 1.

Factors delaying granulocyte death

Factors that enhance survival of granulocytes at the site of inflammation may come from various sources, including immune cells, non-immune cells, pathogens, and the microenvironment. Whereas epithelial cells, T cells, macrophages, and natural killer (NK) cells are sources for various cytokines, granulocytes themselves also produce their own pro-survival factors, suggesting that these factors represent autocrine and paracrine signals, as has been shown for eosinophils [23].

Cytokines and other proinflammatory mediators

The classical granulocyte-regulating cytokines are IL-3, IL-5, and granulocyte macrophage colony stimulating factor (GM-CSF), which may be secreted by different immune and non-immune cells as outlined below. Although eosinophils respond to all three cytokines with an increased lifespan, survival of neutrophils is only enhanced significantly by GM-CSF [24], whereas IL-3 appears to extend only mouse [25] but not human [26] neutrophil lifespan. Basophils, by contrast, functionally respond to all three cytokines, but only IL-3 seems to have a prominent pro-survival effect that depends on the kinase Pim-1, similar to the IL-5-induced survival in eosinophils [26,27].

Another major pro-survival factor for neutrophils is granulocyte-colony-stimulating factor (G-CSF), which is produced by endothelial and epithelial cells, but also by macrophages and other immune cells. Recently, a novel mechanism for the pro-survival effects of G-CSF has been discovered. G-CSF was shown to enhance expression of proliferating cell nuclear antigen (PCNA) in mature neutrophils, which in turn sequesters the proform of proapoptotic caspases and prevents their activation [28].

Other cytokines that have been described to enhance viability of different granulocyte subsets are summarised in Table 2. Of note are two recently described cytokines, IL-33 and thymic-stromal-derived lymphopoietin (TSLP). IL-33, one of the newest additions to the interleukin family, is constitutively expressed by many tissue cells, including endothelial cells, epithelial cells, and keratinocytes, and is frequently upregulated during inflammation [29]. It may act as an alarmin upon cellular damage but can also be actively secreted by dendritic cells. IL-33 has strong effects on eosinophil and basophil, but not on neutrophil, function owing to the lack of expression of the IL-33 receptor ST2 [30,31]. Although eosinophils and basophils respond to IL-33, only eosinophil survival is enhanced by this cytokine, whereas basophil apoptosis seems unaffected [31,32].

TSLP, by contrast, plays a prominent role especially in allergic responses and is mainly produced by non-haematopoietic cells, but may also be secreted by some macrophages, mast cells, and dendritic cells [33]. Eosinophils and basophils are targets of TSLP – neutrophils lack TSLP receptor expression [33,34] – and TSLP has been reported to promote their survival and exacerbates the inflammatory response, although conflicting reports about eosinophil viability exist [34–36].

Although a number of different cytokines, which are elevated during inflammatory responses, enhance granulocyte viability, they usually do not act on all granulocyte types. This allows the immune system to promote production and/or survival selectively of one granulocyte type over the other, depending on which function(s) is/are required. This opens up possibilities for selectively interfering with specific granulocyte subsets under conditions of immunopathology (Table 1).

Cell–cell interactions

An intriguing subject is the interaction of different types of immune cells at the site of inflammation and their effects on cellular function and lifespan. Depending on the cellular