

Neutrophils and Inflammation: Unraveling a New Connection

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Abstract

The inflammatory response can be triggered by a number of different agents. Neutrophils are key players of the inflammatory response and have both protective roles as well as worsening the response. This review article examines potential areas of dampening the inflammatory response.

Keywords: Inflammation; Inflammatory response; Neutrophils; Immunosuppressive therapies

Introduction

Inflammation is acute response to an injury. The inflammatory response can be triggered by different injurious agents. Most commonly, in the setting of critically ill patients, microorganisms and trauma start the inflammatory cascade. Despite the evolution of antibiotics, the inflammatory response can be dampened but not completely inhibited. As a result sepsis continues to be a common problem in the intensive care units. Over the last decade, the incidence of sepsis has increased with a consequent rise in hospitalizations [1]. In fact, the incidence of sepsis per 100,000 in the population has increased from 83 in 1979 to 240 in 2000 [2]. It is expected that the worldwide incidence of sepsis will continue to grow in the face of antibiotic resistance, an increasing proportion of elderly people in the population, and wider use of immunosuppressive therapies [3].

Neutrophils

Neutrophils are known to be 'front-line' defenders [4-7]. Neutrophils are continuously generated in the bone marrow from myeloid precursors. In a normal adult their daily production can reach up to $1-2 \times 10^{11}$ cells [8], controlled by granulocyte colony stimulating factor (G-CSF). G-CSF is a glycoprotein that influences the survival, proliferation, differentiation, and function of mature neutrophil granulocytes and their precursors [9]. During infections, G-CSF becomes essential for tuning the production of neutrophils to meet increased needs. However, the overall production of neutrophils is largely regulated by the rate of apoptosis of neutrophils in tissues.

In order to maintain homeostasis, an equivalent number of senescent neutrophils must be removed from the circulation. Apoptotic or aged neutrophils are cleared primarily by resident tissue macrophages in the liver, spleen, and bone marrow [10,11]. Normal neutrophil turnover in humans is mediated by apoptosis [12], a process that presumably downregulates proinflammatory capacity and microbicidal function and prepares these cells for removal from the tissues by macrophages [13]. Removal of neutrophils by apoptosis is an essential phase in the normal resolution of the inflammatory response, as it prevents damage to healthy tissues that would otherwise occur following necrotic cell lysis. The number of neutrophils in the tissue

increases and, with time, these cells die by apoptosis and are removed by macrophages and dendritic cells (DCs) [8] during an infection.

In addition to normal turnover, phagocytosis initiates a molecular cascade of events that results in accelerated induction of apoptosis in human PMNs [14]. Thus, apoptosis likely represents the terminal stage of inflammation initiated by neutrophil activation.

Turnover of Neutrophils

Neutrophils have shorter life spans than do macrophages and mast cells, and unlike macrophages and mast cells, neutrophils are released into the blood as mature or nearly mature cells devoid of proliferative potential [15]. In healthy individuals, neutrophils have a short half-life, which usually does not exceed more than 12 hours and normally ranges from 1.5-8 hours in the circulation (approximately 1.5 hours in mice and 8 hours in humans) [16-18].

However, under inflammatory conditions, neutrophils become activated and their longevity increases by several fold, which ensures the presence of primed neutrophils at the site of inflammation [19]. The estimated time that neutrophils spend in the circulation increases by tenfold, from 5-10 hours to 5.4 days [17]. When encountering an inflammatory stimulus, apoptosis is avoided for 24 hours or longer [20]. It is thought that a longer lifespan may allow neutrophils to carry out more complex activities, including resolution of inflammation or shaping adaptive immune responses, but their persistence in tissues may lead to bystander cell injury [4]. Thus, in instances where there is an excess of inflammatory stimuli, the prolongation of the neutrophil lifespan can contribute greatly to the morbidity (and possible mortality) associated with inflammation [13,21,22]. A prospective multicenter observational study found that the percentage of neutrophil apoptosis was significantly decreased at 24 hours, 5 days, and 12 days after the diagnosis of septic shock ($14.8\% \pm 13.4\%$, $13.4\% \pm 8.4\%$, and $15.4\% \pm 12.8\%$, respectively; $P < 0.0001$) compared with the control group ($37.6\% \pm 12.8\%$) [23]. This longer lifespan of neutrophils may set the basis for neutrophils to undergo phenotypic and functional changes [24]. Apoptosis provides a mechanism for the clearance of unwanted cells in a variety of situations in which programmed or physiological cell death occurs [20]. It is through this mechanism that neutrophils are able to maintain a homeostatic balance.

Neutrophils may extend their antibiotic activity beyond the life of the neutrophil. The formation of neutrophil extracellular traps (NETs)

is an alternative to death by necrosis or apoptosis. Highly activated neutrophils can eliminate extracellular microorganisms by releasing NETs, which are composed of decondensed DNA and proteins from the cytosol, from granules (which disintegrated at the same time that the nuclei dissolve), and from chromatin (histones) [25]. NETs immobilize pathogens, thus preventing them from spreading but also facilitating subsequent phagocytosis. They are also thought to directly kill pathogens by means of antimicrobial histones and proteases [26,27].

Neutrophils are also involved in a special type of membrane tubulovesicular extensions (TVEs, membrane tethers or cytonemes). TVEs are involved in main aspects of neutrophil physiology. In the bloodstream, the pulling and shedding of membrane tethers from neutrophil cell bodies under shear stress controls the rolling [28]. The increased nitric oxide (NO) concentration in infected lesions induces formation of TVEs in neutrophils. Extracellular binding of bacteria by neutrophil TVEs represents an alternative phagocytotic mechanism to bind and kill pathogens [29-32].

The Death of Neutrophils

In physiological conditions, it is thought that neutrophils are mainly cleared from the circulation in the liver, spleen, and bone marrow [11,33]. In fact neutrophil numbers are controlled by a fine balance between production, retention, mobilization, migration, and clearance. Neutrophil retention in the bone marrow is regulated by the coordinated action of CXCL12 and its receptor CXCR4. Increased CXC-chemokine receptor 4 (CXCR4) expression is seen in aged neutrophils, and this is thought to help direct them back to the bone marrow, where they are then eliminated [4]. Neutrophils can also die in the vasculature and be removed by Kupffer cells (liver-resident macrophages) that live immobilized in the liver vasculature; this applies to both senescent neutrophils and neutrophils that die after fighting infection [11]. Finally, a recent aspect of neutrophil death has described neutrophils as having the ability to break down their nuclear contents and release them as NETs [25,34].

Circulating Neutrophils

In humans, 50-70% of circulating leukocytes are neutrophils [4]. They are the main cells which are recruited to the site of injury during inflammation and are indispensable for defense against injurious stimuli, including intruding microorganisms [7,8,35,36]. In the circulation, mature neutrophils have an average diameter of 7-10 μm , their nucleus is segmented, and their cytoplasm is enriched with granules and secretory vesicles [8].

Different types of neutrophil granules are formed consecutively during their maturation, and they are filled with proinflammatory proteins [6,8,15,37]. The protein constituents of different granules are defined by the timing of their biosynthesis during neutrophil differentiation. Importantly, granule contents are also released according to a hierarchy, with secretory granules being the most readily exocytosed and azurophil granules only undergoing partial exocytosis [38].

There are four main types of neutrophil granules. Azurophilic (primary) granules contain MPO, defensins, and proteinases. Specific (secondary) granules include proteins such as lactoferrin and lysozyme, and gelatinase (tertiary) granules contain leukolysin and matrix metalloproteinase 9 (MMP9; also known as gelatinase B) and

finally quaternary (secretory granules). Azurophilic (peroxidase-positive) and specific (peroxidase-negative) granules can be further subdivided. In humans, azurophilic granules can be differentiated into defensin-high and defensin-low [39].

The various granule subtypes of human neutrophils are formed sequentially during myeloid cell differentiation and differ in their propensity for exocytosis. As a rule, granules formed at late stages of myelopoiesis have a higher secretory potential than granules formed in more immature myeloid cells [39]. Azurophil (peroxidase positive) granules are the first to appear, and are traditionally defined by their content of MPO. They are formed at the promyelocyte stage of neutrophil development. Neutrophils contain four closely related alpha-defensins, which are stored in a subset of azurophil granules. These defensin-rich azurophil granules (DRG) are formed later than defensin-poor azurophil granules, near the time of promyelocyte-myelocyte transition [40,41].

Summary

Neutrophils are key players in the inflammatory process. Perhaps neutrophil granules can be used as potential biomarkers to help quantify the inflammatory response. This would be very useful for patients who present with sepsis or another inflammatory flare of their underlying disease. It would not only help direct therapy but possibly help with overall prognosis. This is a new area of study which could be better explored in a randomized controlled study and could potentially impact on patient care.

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