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Review

Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity

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ABSTRACT

The pathogenesis of many autoimmune diseases is initially based on a redundant or prolonged activation of the innate immune system. It was suggested that an excessive activation of the innate immunity is often the result of a chronic inflammatory process in the organism. This inflammation can be induced by exogenous and endogenous alarm factors, or alarmins. We believe that the recently discovered neutrophil extracellular traps, or NETs, completely meet the criteria of alarmins. This review summarizes current knowledge concerning the general characteristics of NETs, their antimicrobial properties, and their role in the development of chronic inflammatory processes that underlie the pathogenesis of psoriasis and atherosclerosis. Studies on the NETosis can provide the foundation for developing new diagnostic methods and effective treatment of chronic inflammatory and autoimmune diseases.

Contents

1. Introduction

The basis of pathogenesis of many autoimmune diseases initially is a redundant or prolonged activation of the innate immune system, resulting in an excessive activation of adaptive immunity. An excessive activation of the innate immunity is often the result of a chronic inflammatory process in the organism. Exogenous and endogenous factors may induce this process. Exogenous factors include the most conserved structures of microorganisms, i.e. pathogen-associated molecular patterns (PAMPs) [1]. Endogenous factors are usually substances synthesized and secreted from cells under the influence of various damaging agents including PAMPs or appearing as a result of impaired cellular metabolism. These endogenous factors are called alarmins [2,3], and they can cause sterile inflammation.

Alarmins include cytokine IL-1α and amphoterin HMGB1 (high mobility group box 1) that appear at the early and later stages of inflammation, respectively; the neutrophil antimicrobial peptides, cathelicidin

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and defensins; a large group of S100 proteins; factor HDGF (hepatoma-derived growth factor); heat shock proteins (HSPs); uric acid crystals; annexins and highly glycosylated peptides, galectin, thymosin, nucleoline. The products of DNA and RNA decomposition, as well as some structural proteins of the extracellular matrix also belong to alarmins. Alarmins include neutrophil extracellular traps, or NETs, which represent a fundamentally new phenomenon in the physiology of neutrophils [4,5].

Recently, NET release has been found in systemic lupus erythematosus (SLE), ANCA-vasculitis, type II diabetes, atherosclerosis, rheumatoid arthritis, psoriasis, and gout. This review highlights the functional activity of NETs and their role in the development of chronic inflammatory processes underlying the pathogenesis of psoriasis and atherosclerosis. The choice of these diseases is based on the fact that psoriasis is not just a skin disease. A systematic study of various biological markers, including immunological, revealed a close relationship between psoriasis and atherosclerosis, the development of which is associated with the initiation of chronic inflammation induced by various exogenous and endogenous alarmins [6–8].

2. General characteristics of NETs

In 2004, Brinkmann and colleagues [4] discovered that neutrophils were able to kill pathogens outside the cells by releasing chromatin fibrils, or neutrophil extracellular traps. Since neutrophils lose their viability in the process of trap formation, Steinberg and Grinstein [9] denoted this form of neutrophil cell death as "NETosis" in 2007. It should be noted that trap formation has also been shown for eosinophils [10], mast cells [11], and monocytes/macrophages [12]. Since chromatin is released into the extracellular milieu not only by neutrophils but also by other types of cells, the broader term used for this mechanism is ETosis (from Extracellular Traps, ETs).

NETs have a unique ultrastructure. Their framework is formed by chromatin filaments ~15–17 nm in diameter [4] consisting of modified nucleosomes [13]. This framework is dotted with globular structures about 50 nm in diameter [4,5,14]. Chromatin filaments are composed of DNA and histones, where histones account for 70% of the total proteins of the traps. The composition of filaments includes nuclear protein amphoterin HMGB1 (high-mobility group box 1), which is a part of the chromatin of the intact cell. Globular structures consist of components of primary and secondary granules of neutrophils, such as neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, proteinase 3, bactericidal proteins: BPI (cationic bactericidal/permeability-increasing protein), calgranulin, α-defensins, lactoferrin, a fragment of the protein cathelicidin hCAP18 — the peptide LL-37, and pentraxin PTX3. Among the components of the tertiary granules, NETs include matrix metalloproteinase-9 (MMP-9) and peptidoglycan recognition protein-S (PGRP-S) [15–17].

Surprisingly, NETs may not only have the morphology of elongated thin filaments. They can also be cloud-like structures that occupy a 10–15-fold greater area compared to the initial cell size.

NETs are formed because of a unique form of cell death: an initial loss of all intracellular membranes is followed by the disintegration of the cytoplasmic membrane. To date, little is known about the mechanisms of NETosis. Nevertheless, neutrophils are known to undergo major morphological modifications in the process of NETosis. Several minutes after activation, the cells lie flat, being tightly attached to the substrate. During the next hour the nucleus loses its lobules, chromatin is decondensed, and the inner and outer leaflets of the nuclear membrane separate. The disintegration of the granules occurs simultaneously. Within another hour, the nuclear membrane breaks up into separate vesicles, while the nucleoplasm and cytoplasm merge into a homogenous mass. Finally, the cells become rounded and seem to be contracted until the cytoplasmic membrane is broken; then the cell contents are excreted to the exterior and form bundles of thin filaments, i.e. NETs.

On the molecular level, NET formation is a gradual process with several successive steps: (1) ROS generation; (2) transport of neutrophil elastase, and, subsequently, myeloperoxidase from the granules to the nucleus; (3) histone modification, and, finally, (4) disruption of cytoplasmic membrane and release of chromatin. Of much interest are the details of these steps, because they constitute a unique mechanism of cell death, i.e. NETosis.

It has been shown that ROS are necessary for NETosis. The most powerful inducer of NETosis is phorbol 12-myristate 13-acetate (PMA), and it is also the most potent inducer of ROS generation. ROS are probably responsible for the oxidative modification of DNA, proteins and other macromolecules, which make them more susceptible to the influence of neutrophil enzymes [18]. In neutrophils, ROS are formed during a "respiratory burst" involving the NADPH oxidase complex [19]. This multicomponent enzyme complex is assembled during cell activation on the cytoplasmic membrane and on the membranes of the specific granules of neutrophils. It performs the electron transfer from NADPH located in the cytoplasm to molecular oxygen across the membrane. The involvement of ROS in NET formation has been proven pharmacologically using diphenyleneiodonium and other inhibitors of NADPH oxidase, which nearly completely abolish NETosis [20]. Besides, research on the neutrophils of patients with chronic granulomatous disease (CGD) revealed that this pathology is due to mutations in NADPH oxidase subunits resulting in the assembly of a nonfunctional or lowly functional enzyme complex, which is unable to synthesize ROS. People with such mutations suffer from recurrent infections in their lifetime [21,22], and their neutrophils do not form neutrophil traps [20]. However, it has been shown that the addition of H_2O_2 to the neutrophils of CGD patients restores the ability to release NETs [20].

Molecules involved in signal transduction from the receptors to NADPH oxidase were revealed by rigorous inhibition analysis carried out in Zychlinsky's laboratory [23]. It has been shown that the activation of NETosis by PMA is accompanied by the induction of the Raf/MEK/ERK signaling pathway [23], as well as the Rac2 (a small GTPase of the Rho-family)-mediated pathway [24].

Another feature of NETosis is the loss of chromatin segregation into eu- and heterochromatin [20]. This process involves neutrophil elastase and myeloperoxidase, the enzymes of primary (azurophilic) granules. Both enzymes move from the granules into the nucleus at the earliest stages of NETosis. NE is the first to be transported into the nucleus, where it catalyzes the cleavage of the linker histone H1 and modifies the core histones [25]. It has been shown that elastase is extremely important for trap formation, because mice deficient in this enzyme were incapable of producing NETs [25]. MPO migrates into the nucleus later, and its function is associated with intensification of chromatin decondensation, probably due to the synthesis of hypochlorous acid [25]. It should be noted that patients with mutations in the *MPO* gene cannot form valid NETs [26].

In addition to partial cleavage of histones by elastase and MPO, another modification intensifies chromatin decondensation. Peptidylarginine deiminase 4 (PAD4) induced in the neutrophil after proper activation catalyzes deimination of arginine residues that yields citrulline in three out of the four core histones, which results in their weaker binding to DNA. There is evidence that histones are citrullinated in decondensed chromatin [27–29]. The role of this process in NETosis was demonstrated pharmacologically for cell lines forming a limited number of NETs.

It has been shown that autophagy is necessary for NETosis, and this mechanism follows NADPH oxidase activation in the information transfer pathway [30].

Recently, a unique mechanism of NET formation was described, where the cytoplasmic membrane remained intact and the cells preserved viability [31]. Such a mechanism of NET formation was denoted vital NETosis, while a conventional NETosis resulting in cell death was called suicidal NETosis. It should be noted that vital NETotic neutrophils were capable to carry out the main functions, chemotaxis and phagocytosis [32–34].

The main difference between vital NETosis and suicidal NETosis is concerned with the nature of their inducers. Vital NETosis is induced