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Understanding the molecular mechanisms of NETs and their role in antiviral innate immunity

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ABSTRACT

Polymorphonuclear neutrophils (PMNs) are the most abundant cells in the context of innate immunity; they are one of the first cells to arrive at the site of viral infection constituting the first line of defense in response to invading pathogens. Indeed, neutrophils are provided with several defense mechanisms including release of cytokines, cytotoxic granules and the last recently described neutrophil extracellular traps (NETs). The main components of NETs are DNA, granular antimicrobial peptides, and nuclear and cytoplasmic proteins, that together play an important role in the innate immune response. While NETs were first described as a mechanism against bacteria and fungi, recently, several studies are beginning to elucidate how NETs are involved in the host antiviral response and the prominent characteristics of this new mechanism are discussed in the present review.

Contents

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1. Introduction

Neutrophils are essential components of the innate immune response against pathogens. Since neutrophils are one of the first and most abundant cell population to reach the site of infection, it was postulated that these cells are implicated in the antiviral immune response. However, the protective effect of neutrophils during viral infection has been controversial since it had been reported that they mediate both beneficial and detrimental effects on the host (Drescher and Bai, 2013; Jenne and Kubes, 2015; Mantovani et al., 2011). In addition, the detection of positive and negative sense viral RNAs in human and mouse neutrophils demonstrated that several viruses can replicate in these cells (Drescher and Bai, 2013). To kill pathogens, neutrophils use a number of strategies such as phagocytosis, degranulation and the recently described formation of neutrophil extracellular traps (NETs). The generation of NETs was initially described as a microbicidal mechanism that is part of the arsenal of neutrophils. However, we now know that this mechanism is not limited to these cells, since other cells are also able to form extracellular traps (ETs), such as monocytes, macrophages and mast cells (MCs), eosinophils and dendritic cells (Jenne and Kubes, 2015). NETs are structures that contain various components that favor the capture and elimination of pathogens such as bacteria, fungi and parasites (Goldmann and Medina, 2012). Since the discovery of NETs, recent studies have been concerned with the role of NETs in viral pathogenesis (Jenne and Kubes, 2015). Here we will review recent findings toward the understanding NETs, how they are formed and how they function, and also discuss their importance in viral infections.

2. Neutrophils: functions and their role in inflammatory response

Polymorphonuclear neutrophils (PMNs) play a major role in the early inflammatory response to viral infection or injury. Their function depends on the maturation state in which they are released from the bone marrow into the blood circulation system, where they have a half-life of between 12 h and 5 days depending on the stimuli or challenges to which they are exposed (Colotta et al., 1992; Kim et al., 2011; Pillay et al., 2010). The shorter or longer half-life will determine whether the inflammatory response will extend or terminate.

In bone marrow, PMNs can be divided into three cell groups: stem cells including the undifferentiated hematopoietic stem cells (HSCs) CD34+; mitotic cells containing granulocyte progenitor cells in the process of proliferation and differentiation; and the group of post-mitotic cells consisting of fully mature and differentiated neutrophils that constitute ae reservoir in bone marrow (Summers et al., 2010). The main regulator of granulocytopoiesis—granulocyte colony-stimulating factor (G-CSF) promotes and controls neutrophil production under steady and infectious conditions. The production of G-CSFs, described almost three decades ago and produced in part by T cells, has a potent, rapid and specific effect on the proliferation of granulocytic progenitors and promotes neutrophil differentiation (Cohen et al., 1987; Richards et al., 2003). Neutrophil recruitment to the specific site of inflammation plays a critical role in the innate response and requires the formation of a complicated and organized signaling complex with the participation of chemoattractant molecules, membrane receptors and ligands. The close collaboration between these molecules begins with the intracellular signaling that allows the neutrophils to leave the bloodstream, while the signals mediated by interaction with tissue-resident cells facilitates their migration (Nauseef and Borregaard, 2014). After passing through the endothelium, neutrophils follow a chemokine gradient released from resident tissue cells. Among the first discovered chemokines, interleukin 8 (IL-8 or CXCL8), produced by activated monocytes, macrophages, mast cells, endothelial cells and neutrophils (Ghasemi et al., 2011), is one of the most important chemokines in neutrophil recruitment to the inflammation site (Rollins, 2009; Van Damme et al., 1989). In the murine model it was observed that neutrophils synthesize and secrete CXCL1 (homologous to human IL-8) and CXCL2 after being stimulated with lipopolysaccharide (LPS) and that this regulation is performed through Toll-like receptors (TLR)- 2 and TLR-4 in a MyD88- and TRIF-dependent manner (De Filippo et al., 2013; De Filippo et al., 2008).

Once at the inflammation site, neutrophils carry out key functions to eradicate infectious agents or inflammatory processes, including phagocytosis and killing microorganisms in phagosomes. To achieve this, neutrophils produce reactive oxygen species (ROS) through the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system (Arruda and Barja-Fidalgo, 2009), and release cytotoxic granules with antimicrobial proteins such as defensins, cathelicidin, lactoferrin, elastase and myeloperoxidase (MPO) (Dale et al., 2008; Klebanoff, 1968). In addition to the elimination of bacteria by phagocytosis, neutrophils have developed different mechanisms allowing them to actively participate in host defense, such as extracellular microbicidal mechanisms, including release of cytotoxic granules and NETs. The state of neutrophils with NET formation is known as NETosis, a phenomenon described a decade ago as a novel mechanism of cell death with microbicidal properties (Brinkmann et al., 2004), that will be discussed below.

Inflammation is a pathophysiological response to infection or tissue damage, and to be carried out, cells of the innate immune response launch a restoration program of homeostasis consisting of different steps. First, phagocytes and specialized antigen-presenting cells (macrophages, monocytes and dendritic cells) recognize alarming molecular signals generated by tissue damage and/or invading microorganisms through pattern recognition receptors (PRRs), resulting in the production of pro-inflammatory cytokines and chemokines, including TNF-α, IL-6, CXCL1, CXCL2 and IL-8. These molecules stimulate neutrophil recruitment to the damaged site, leading to the next stage in which recruited neutrophils release granule proteins, such as cathelicidin LL-37 and azurocidin, as well as the chemokines CCL3, CCL4 and CCL20, promoting the extravasation of inflammatory monocytes and neutrophils to the damaged site (Rigby and DeLeo 2012; Filep and El Kebir, 2009; Fadok et al., 1998).

Once monocytes, macrophages and neutrophils have entered the infection site and caused elimination of the injurious agent, the current inflammatory response must be resolved to avoid excessive tissue damage (inflammatory resolution). Among the mechanisms involved in abrogating the inflammatory status is the activation of spontaneous apoptosis in neutrophils. In this case, neutrophils undergo changes in their membrane composition, specifically in the negative charges of their surface as well as release of lipids, proteins and nucleic mediators (Filep and El Kebir, 2009; Rigby and DeLeo, 2012). These changes function as signals to attract scavenger cells and macrophages that handle removal of apoptotic neutrophils. Finally, the uptake of apoptotic bodies acts as a stimulus for macrophages which release mediators that suppress the inflammatory response, such as IL-10 and transforming growth factor-β (TGF-β) (Fadok et al., 1998). In neutrophils, the inflammatory response can be triggered by TLRs. Previous studies reported that human neutrophils express TLR-1, -2, -4, -5, -6, -7, -8, -9 and -10 and that the stimulation of TLRs, receptors such as NOD and dectin-1 induce the production of pro-inflammatory cytokines (Moreno et al., 2014; Prince et al., 2011). TLR stimulation with the respective agonists induces expression of L-selectin, decreases chemotaxis, and increases phagocytosis, ROS production