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Chapter 2

Beneficial and Deleterious Effects of Neutrophil Extracellular Traps on Infection

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Additional information is available at the end of the chapter

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Abstract

Polymorphonuclear neutrophils (PMNs) are the most abundant leukocytes in the blood and are considered as the first line of innate immune defence against infectious diseases. However, PMN cells have a crucial function in both innate and adaptive immune responses. Neutrophils have several mechanisms to control pathogens, and one of them is their capability to form neutrophil extracellular traps (NETs) that may control infection. NETs have the capacity to trap microorganisms, kill them, or avoid their dissemination. The aim of this chapter is to provide a comprehensive review on NETs, the cells that produce them, and some of the mechanisms involved in their formation, their role in the immune response, and the pros and cons of NETs, focusing mainly on infectious diseases.

Keywords: neutrophil extracellular traps (NETs), neutrophils, bacteria, viruses, infectious diseases

1. Introduction

The polymorphonuclear neutrophils (PMNs), first reported by IIya IIych Mechnikov, better known as Élie Metchnikoff, are the most abundant leukocytes (60%) in the blood. These PMNs are considered as the first line of innate immune response against infectious agents [1]. Later on, Carl Friedrich Claus suggested the term of phagocytosis for the function of these cells. Studies aimed at the fully understanding of their properties and functions in controlling a variety of pathogens are still in progress. Research on neutrophils has focused on their

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phagocytic capacity and, more recently, on their role as neutrophil extracellular traps (NETs) forming cells, in innate and adaptive immunity.

When neutrophils fail to kill invading pathogens by the classical phagocytosis mechanism, PMNs can accomplish this function by neutrophil extracellular traps (NETs), a process reported as a novel form of cell death called NETosis, which is dependent of the generation of reactive oxygen species [2–5]. Neutrophils forming NETs have been demonstrated by activating neutrophils with phorbol myristate acetate (PMA), interleukin 8 (IL-8), lipopolysaccharide (LPS), or under contact of neutrophils with Gram-negative and Gram-positive bacteria.

NETosis induction has also been described for viral infections, and some of the signaling pathways involved have been analyzed, finding the involvement of pathogen-associated molecular patterns (PAMPs), TLR-4, TLR-7, and TLR-8. Rodríguez-Espinosa et al. have shown that NETs formation takes place in two separate metabolic steps: the first one involves chromatin decondensation, which is independent of external glucose and glycolysis, whereas the second, which involves the chromatin release, is a process that is dependent on external glucose and glycolysis [6].

2. Understanding the process of NETs formation

The neutrophil extracellular traps (NETs) structures were described as another type of neutrophil cell death, different from apoptosis and necrosis. The research field on NETs has steadily been growing since 2004, when Brinkmann et al. reported for the first time this new function of activated neutrophils, demonstrating, by electron microscopy, that, when neutrophils are in the presence of bacteria, fungi, protozoa, or viruses, they acquire the capacity to form fibrillary structures, resembling nets or webs. These structures are composed mainly of nuclear material, chromatin fibers with diameters of 15–17 nm containing DNA decorated with neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, proteinase 3 (PR3), high-mobility group protein B1 (HMGB-1), tryptase or antimicrobial peptide LL37, histones, and cytoplasmic proteins such as histones H1, H2A, H2B, H3, H4, G, lactoferrin, and gelatinase, among others [7].

Two mechanisms for the formation of NETs have been described: the suicide or lytic and vital NETosis [8]. In the first case, NETs release results from the activation of PMN by IL-8 or chemical compounds, such as phorbol myristate acetate (PMA). PMA activates neutrophils through the protein kinase C (PKC) and follows the Raf-MEK-ERK mitogen-activated protein kinase signaling pathway; the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase induces the translocation of elastase from the cytosolic granules to the inner nucleus, helping the rupture of the chromatin through histones. Induction of NETs with PMA by this mechanism can be observed from 30 min post-activation and, by 6–8 h post-activation, a high number of extracellular traps (ETs) are well formed (**Figure 1**).

In contrast, vital NETosis has been demonstrated following pathogen recognition by host pattern recognition receptors (PRRs). Gram-negative bacteria products, such as lipopolysaccharide

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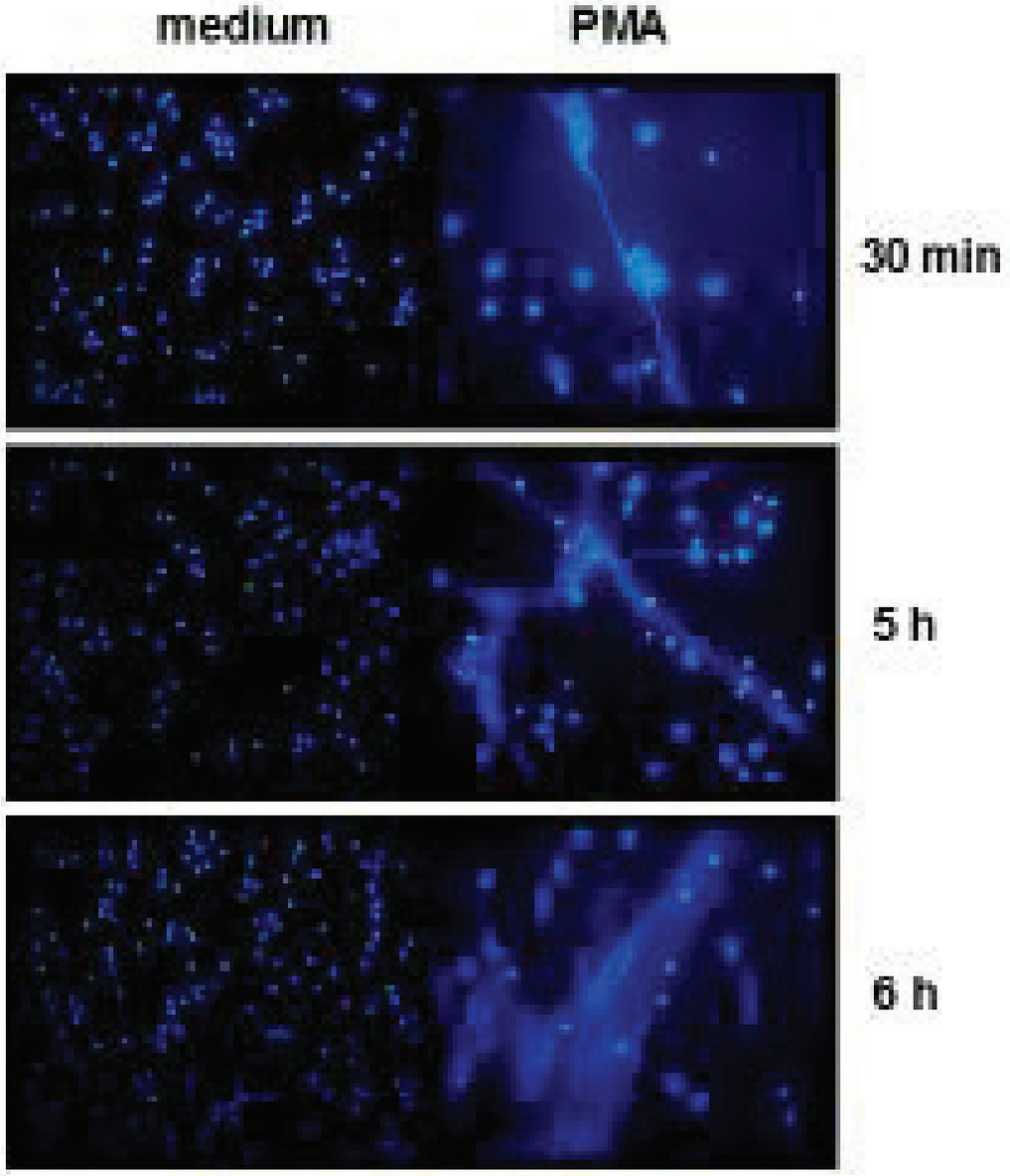


Figure 1. Human peripheral blood neutrophils non-activated and activated with PMA (100 ng/ml) for different lengths of time. Neutrophil extracellular traps formation starts by 30 min post-activation; extracellular traps are more extended by 6 h post-activation (photographs taken by Moreno-Altamirano).

(LPS), activate neutrophils, by the ligation of TLRs (TLR-4 in the case of LPS), inducing the liberation of NETs. In the case of Gram-positive bacteria, the complement receptor 3 (CR3) and TLR-2 are required to induce vital NETosis; platelets are also inducers of vital NETosis, through CD11a. This mechanism maintains the external membrane integrity and thus the function of neutrophils, until cells are devoid of nucleus [7, 8].

A third mechanism for the induction of NETs, recently reported, is through autophagy [9, 10]. It is worth mentioning that neutrophils are not the only cells that form extracellular traps (ETs), and other immune cells, such as mast cells, eosinophils, and macrophages, can also

release ETs. Although the molecular principles underlying the formation of ETs by mast cells [11], eosinophils [12], and monocytes/macrophages [13] are similar to those observed in neutrophils, there are some notable disparities. The most remarkable mechanism of ET formation has been described in eosinophils. In these cells, ETs are formed by both nuclear and mitochondrial DNAs, in a reactive oxygen species (ROS)-dependent manner.

Neutrophil extracellular traps are able to capture microorganisms trap microorganisms, killing them or not, this much depends on the type of pathogen involved. NETs are produced by the neutrophils of mice, humans, and some other animals, and can be induced by chemical compounds, bacteria, fungi, protozoa, and viruses. The role of NETs in viral infections is not yet clear. However, some viruses induce the release of NETs [14, 15].

While some viruses are immobilized and inactivated by NETs, others such as HIV induce the production of an IL-10-like protein that inhibits the formation of NETs [15], and dengue virus inhibits PMA-induced formation of NETs. Interestingly, neutrophils seem to be arrested at the chromatin decondensation step, failing to liberate NETs, thus suggesting a metabolic-related mechanism of NETs inhibition [16].

Controversy surrounding neutrophil extracellular traps as a host defense mechanism makes it necessary to analyze how NETs limit the growth of various infectious agents, whereas, apparently, they have no effect on others. On the other hand, how NETs may cause damage and autoimmune diseases also needs to be investigated.

3. Neutrophil extracellular traps in bacterial infections

Several mechanisms have been proposed to explain how NETs control bacterial infection. NETs bind to both Gram-negative and Gram-positive bacteria, precluding bacterial mobilization and dissemination, and some bacteria are killed extracellularly by NETs, due to their high content of serine proteases [17]. Some bacteria and their interaction with NETs are summarized as follows:

Bordetella pertussis, the causative agent of pertussis or whooping cough, is a Gram-negative aerobic bacterium that infects the respiratory tract and inhibits the host´s immune system by mean of its virulent factors, such as pertussis toxin, filamentous hemagglutinin, pertactin, fimbria, and tracheal cytotoxin. The pertussis toxin inhibits G protein coupling that regulates the adenylate cyclase-mediating conversion of ATP to cAMP. This event induces macrophages and neutrophils to convert the ATP to cAMP by intracellular eukaryotic calmodulin, causing disturbances in cellular signaling mechanisms and thus preventing phagocytosis and an efficient control of the pathogen. The formation of NETs induced by *B. pertussis* is NADPH oxidase dependent [18].

Escherichia coli, the causative bacteria of several pathologies, including bacterial sepsis, is a Gram-negative bacterium. NETs formation helps to control infection by trapping and killing the bacteria and avoiding dissemination to other organs. The proposed mechanisms for the formation of NETs depend on the bacteria strain and its pathogenesis. In the case of *E. coli*

involved in liver sepsis, the infection can be controlled by histones H2B or by activating the intravascular NETs release through the integrin lymphocyte function-associated antigen 1 (LFA-1) [19, 20].

Klebsiella pneumoniae, the common cause of pneumonia, is caused by this aerobic Gram-negative bacillus. The role of NETs in the killing of *K. pneumoniae* has been investigated; this bacterium is not sufficient to induce NETs in neutrophils *ex vivo*, but it is in the lungs of a murine model. Adenosine A2B receptor deficiency improves survival and enhances bacterial killing and clearance due to NETs formation [21]. In addition, TREM-1 also mediates NETs formation, leading to a bactericidal effect and the control of infection [22].

Leptospira interrogans is the causative agent of leptospirosis. The pathogen spirochetes Gram-negative belongs to the Leptospiraceae family and to the genus Leptospira. Leptospirosis is an emerging zoonotic disease, affecting animals and humans in the world, but most frequently in tropical and subtropical countries. This disease is associated with exposure of individuals to wild or farm animals. Scharrig et al. [23], demonstrated for the first time the induction of NETs in human *ex vivo* and murine *in vivo* models, when incubating human neutrophils with Leptospira interrogans LI-130 (LIC). This research group observed that the bacteria number, the pathogenicity, and viability were relevant factors for induction of NETs; however, the motility of bacteria was not. Entrapment of LIC in the NETs resulted in Leptospira death. Pathogenic, but not saprophytic, Leptospira exerted nuclease activity, thus degrading the DNA, concluding that formation of NETs was dependent on bacterial concentration, pathogenicity, and viability, but not motility, and that NETs could trap and kill *Leptospira interrogans* [23].

Mannheimia haemolytica, the causative agent of bovine respiratory disease complex (BRD), is a Gram-negative bacterium that induces a severe pleuropneumonia in bovine animals, where neutrophils play a key role in the pathogenesis. Extracellular traps are induced in neutrophils and macrophages exposed to the bacteria or to their virulent factor, leucotoxin (LKT) [24].

Mycobacterium bovis, the etiological agent of bovine tuberculosis, is a Gram-positive bacterium, with a worldwide distribution, easily transmitted to bovine animals and to humans. The extracellular traps formation has been demonstrated in neutrophils and macrophages. Neutrophils can sense the size of pathogens, and based on their size, neutrophils are induced to undergo necrosis, apoptosis, or NETosis [25].

Mycobacterium tuberculosis is the causative agent of tuberculosis. Ramos-Kichik et al. showed that both *M. tuberculosis* and *Mycobacterium canetti* can induce NETs, which trap but not kill these mycobacterial species [26]. On the other hand, the mycobacterium-derived early secretory antigenic target protein of 6 kDa (ESAT-6) can induce the formation of NETs in *M. tuberculosis*-infected neutrophils [27].

Pseudomonas aeruginosa, the causative agent of the cystic fibrosis lung disease, is a Gram-negative opportunistic bacterium. The formation of NETs in the context of *P. aeruginosa* is controversial, and evidence that NETs may have a major anti-*P. aeruginosa* activity must be clarified [28].

Salmonella typhimurium, a Gram-negative bacterium, induces the release of NETs, and some of their components, such as histones (H2), have bactericidal activity, whereas others, such as elastase, can degrade virulence factors, as in the case of the alpha toxin [7, 29].

Shigella flexneri, a Gram-negative bacterium, induces the release of NETs. *S. flexneri* is trapped by NETs and killed via the neutrophil elastase; virulence factors such as IcsA and IpaB are degraded by the neutrophil elastase [7].

Staphylococcus aureus is some Gram-positive bacteria that cause sepsis. The role of NETs in controlling a *S. aureus* infection could be through the antimicrobial proteins associated to these, the bactericidal effect of H2 histones, the antimicrobial action of the cathelicidin LL-37, and neutrophil proteases that decrease the secretion of the alpha-toxin (α-toxin). The virulence factors LukGH and PVL help to induce the release of NETs. The *S. aureus*-induced release of NETs is an NADPH oxidase-independent process [30].

Staphylococcus epidermidis belongs to the group of coagulase-negative straphylococci. It is a quite common colonizer of healthy mice and human skin. It is a part of "normal" skin flora and plays a beneficial role in cutaneous niche. However, in immunocompromised patients, there is a high risk of developing infection mainly due to catheters use in hospitals. The exoprotein of *S. epidermidis*, the delta-toxin, PMSs (Phenol-Soluble Moduline-gamma) cooperates with host antimicrobial peptides to help kill pathogens of the group A of Streptococcus (GAS). In 2010, Cogen et al. [31] reported that the exoprotein phenol-soluble-moduline -gamma (PSMs) (δ-toxin) can induce NETs formation. The authors demonstrated a direct binding of δ-toxin to LL-37, CRAMP, hBD2, hBD3, as well as DNA.

Streptococcus spp. are Gram-positive bacteria that include non-pathogenic commensal strains and highly virulent pathogenic strains. The pathogenic strains express virulent factors that allow them to evade the immune system. *Streptococcus pneumoniae* infection leads to pneumonia and invasive diseases such as meningitis and bacteremia, whereas *Streptococcus pyogenes* is the major causative agent of Severe Group A Streptococcal Infections. *S. pneumoniae* and *S. pyogenes* induce the formation of NETs. However, these bacteria have evolved mechanisms that allow them to modulate the formation of NETs. Neutrophils, on the other hand, have evolved a NETs release mechanism in response to *Streptococcus*-derived virulence factors. The *S. pyogenes* virulent factor M1 decreases the induction of NETs while conferring bacterial resistance to be killed by NETs. The *S. pyogenes*-derived M1 exotoxin induces the formation of NETs, by associating with fibrinogen and forming a complex that stimulates neutrophils. Formation of NETs contributes to the pathogen elimination [32].

In summary, this review shows that in response to bacterial stimuli, neutrophils get activated and form NETs that may trap and kill invading bacteria. Besides the "classical" way of clearing pathogens by phagocytosis and intracellular exposure to bactericidal compounds, this novel mechanism of neutrophil extracellular killing plays an important role in primary host defense. Moreover, knowledge on the mechanisms of bacterial adaptation to evade the immune system could be used in the medical practice. For instance, DNases inhibitors can be used as potential therapeutics, to prevent degradation of NETs by Group A Streptococcus DNases. In the future, therapeutics aimed at the maintenance of NETs could be used to help clear bacterial infections.

4. Neutrophil extracellular traps in parasitic infections

Neutrophil extracellular traps have been broadly studied in regard to bacteria. The role of NETs against protozoa, however, has just recently been analyzed. Protozoa can induce NETs in neutrophils and macrophages, and knowledge on the mechanisms at play is just emerging.

In 2011, Abdi Abdallah [33] reported that human neutrophils produce NETs in response to stimulation with *Plasmodium falciparum* trophozoites, *Leishmania braziliensis*, and *Toxoplasma gondii* tachyzoites. *In vitro* experiments have demonstrated the presence of NETs upon bovine neutrophils stimulation with *Eimeria bovis* sporozoites, in human neutrophils after stimulation with promastigotes of *Leishmania donovani, Leishmania major, Leishmania chagasi, or L. amazonensis* amastigotes. A brief description of the mechanism involved in protozoa-induced NETs formation is next described.

Toxoplasma gondii is an obligated intracellular parasite that causes toxoplasmosis in immunocompromised individuals. In immunocompetent individuals, however, the immune system usually keeps the parasite from causing illness. *Toxoplasma gondii* tachyzoites induce the release of NETs by activating the MEK-ERK signaling pathway. NETs can trap *Toxoplasma gondii* tachyzoites, eliminating about 25% of them as parasite trapping avoids their dissemination [34].

Plasmodium falciparum, an intracellular parasite, causes malaria. It is estimated that this parasite infects between 215 and 659 million humans per year, worldwide. Malaria is transmitted to humans by the bite of Anopheles mosquitoes. *P. falciparum* sporozoites develop into merozoites and enter into erythrocytes. Studies conducted in Nigerian children infected with *P. falciparum* showed NETs structures with trapped trophozoites, and in their blood, infected and non-infected erythrocytes were also observed [35–37].

Eimeria bovis. This parasite is the causative agent of enteritis in cattle, and NETs formed are released upon stimulation with *E. bovis* sporozoites. This parasite stage of *E. bovis* seems to be a better inducer of NETs than PMA. NETs have been shown to diminish infection by parasite immobilization and also by parasite killing, although to a lesser extent [38, 39].

Leishmania spp. These protozoal parasites are the causative agents of leishmaniosis, and the leishmaniosis model has been quite useful in studies on the role of NETs at the early stages of the disease. The promastigote has been identified as the main parasite stage as inducer of NETs. Promastigotes and amastigotes numbers diminish upon NETs release. Histones H2A and H2B are the main inducers of NETs, and these are highly toxic for the parasite. The promastigote form of the parasite can evade the NETs by means of its 3′ nucleotidase, enzyme that degrades the DNA, allowing *Leishmania* spp. to escape from being killed by NETs [40].

In 2015, Rochael et al. analyzed the role of reactive oxygen species, neutrophil elastase, myeloperoxidase, and the PAD4 enzyme in the formation of NETs by *L. amazonensis* promastigotes, in human cells. These authors observed that *Leishmania* promastigotes promote a redox disbalance in neutrophils. The exposure of neutrophils to H_2O_2 induces histone deamination

mediated by PAD4, and the redox disbalance takes place independently of the parasite viability, thus suggesting that *Leishmani*a induces the production of ROS through an NADPH oxidase-dependent mechanism [41].

Leishmania as well as *Staphylococcus aureus* induces the release of NETs by an early and rapid mechanism, through an ROS-independent pathway, which is inhibited by an elastase inhibitor and, in contrast to classic NETosis, is not affected by chloramidina. PAD4 activity is only relevant during classic NETosis. Promastigotes viability after treatment of parasites with a NETs-rich supernatant, obtained from either the early and rapid or the classic pathways, shows a reduction of about 42% [41].

As previously described, the interaction of *Leishmania amazonensis* with human neutrophils leads to the release of NETs, which trap and kill the parasite. However, the signaling pathways leading to *Leishmania*-induced NETosis are still under study. However, it has been shown that PI3K, independently of protein kinase B, has a role in parasite-induced NETosis. The main PI3K isoforms involved are PI3Kγ and PI3Kδ. Activation of ERK downstream of PI3Kγ is necessary to trigger an ROS-dependent parasite-induced NETosis. Pharmacological inhibition of protein kinase C also significantly decreases parasite-induced NETs release. Intracellular calcium, regulated by PI3Kδ, represents an alternative ROS-independent pathway of NETosis stimulation by *L. amazonensis*. Finally, intracellular calcium mobilization and reactive oxygen species generation are the major regulators of parasite-induced NETosis. These results contribute to a better understanding of the signaling behind *Leishmania*-induced NETosis [42].

Entamoeba histolytica. This protozoan parasite causes amebiasis, amoebic colitis, and hepatic abscess. Since this parasite is too large to be phagocytosed, Avila et al. [43] analyzed the possibility that this parasite induces the formation of NETs. These authors demonstrated that the amoeba lipopeptidophosphoglycan induces NETs in a dose-dependent manner. NETs can be readily observed 15 min after stimulation; however, by 1 h at a 1:20 infection ratio, NETs occupy a whole microscopic field. NETs induction depends on trophozoite integrity; 30 min after contact with NETs, trophozoites show no changes in size or morphology, and this contact does not have any effect on viability or growth at any time of incubation. On the other hand, it was observed that *E. histolytica* is resistant to cathelicidin LL-37. Resistance to NETs exposure was also studied upon addition of a proteases inhibitor, resulting in that proteases are not responsible for trophozoite resistance to NETs. However, the use of ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), a divalent anion chelant, had a deleterious effect in the growth of amoebas that were in contact with NETs, suggesting that trophozoites may have DNAse activity, responsible for its resistance to NETs [43].

Ávila et al. demonstrated that parasite growth could only take place in the absence of a calcium chelant, since enzymes such as trophozoite DNAsas require calcium. This provides an example of NETs inhibition by parasite-produced enzymes. *Entamoeba histolytica* is one of the main parasites that cause stomach diseases worldwide. It causes intestine and liver invasion, associated with the recruitment of large amounts of neutrophils at the early stages of infection [43].

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5. Neutrophil extracellular traps in fungus infection

5.1. *Aspergillus fumigatus* and *Aspergillus nidulans*

The *Aspergullus fumigatus* species is isolated in 80% of invasive aspergillosis patients. Chronic granulomatosis disease patients, whose cells are not able to undergo respiratory burst, are highly susceptible to infection by fungus of the *Aspergillus* genus such as *A. nidulans*. This indicates the important role of the host respiratory burst, which is also involved in the formation of NETs.

Recent reports highlight the importance of glucosaminoglycans (GAG) in *A. fumigatus* virulence. GAG helps the formation of biofilms and purified soluble GAG induces NK cell-mediated apoptosis of neutrophils, *in vitro*.

Fungus resistance to neutrophil-mediated killing positively correlates with the amount of cell wall-associated GAG. Fungus GAG content functions as the analog of bacterial capside, enhancing resistance to NETs. Although the mechanism by which exopolysaccharides mediate resistance to NETs has not been defined, it is suggested that GAG may inhibit hyphae-NETs binding, perhaps due to the repulsion between the *Aspergillus* exopolysaccharide positive charges and the positive charges present in the NETs antimicrobial peptides and histones [44, 45]. *Aspergillus* induces respiratory burst through its glycosaminoglycans that activate the NAPDH oxidase system, yielding ROS and activating classic NETosis activation [44].

5.2. *Candida albicans*

In 2006, Urban et al. showed that NETs can kill *Candida albicans* in any of its two forms, yeast, which is the proliferating form, or the filamentous, which is the invasive and tissue destructive form. This was corroborated by means of electronic microscopy which showed NETs and *C. albicans* hyphae co-localization, which suggested that hyphae are trapped by NETs, thus controlling the infection [46, 47].

Experiments aimed at analyzing the effect that PMA-activated NETs have on *C. albicans* showed that 20–30% of fungus dies after exposure to NETs [46].

The analysis of the components present in the neutrophil granules that may be responsible for the killing of *Candida albicans* showed that histones are not accountable for this. It was determined that human Neutrophil Granular Extract (hNGE) is responsible for the fungus death, in a dose-dependent manner. These granules contain Bactericidal/permeability-increasing (BPI) protein lactoferrin, and defensins. It appears that the release of NETs is related to the microorganism cell wall composition; the binding of microorganisms by NETs is mediated by ionic forces and thus, the fact that the *Candida* wall contains numerous proteins with phosphodiester bonds with negative charges makes it likely that they bind the positive charges of proteins and histones present in NETs [48].

Kenno et al. analyzed the induction of NETs by *Candida albicans*, and they corroborated that the distinctive forms of *Candida albicans*, hyphae or yeast, may induce NETs. These authors

found that hyphae induce higher amounts of NETs than the yeast form, after 4 hours of incubation. *Candida albicans* hyphae stimulate cells through autophagy but not ROS, whereas the yeast form induces NETs through autophagy and ROS. *C. albicans β-glycans* induce NETosis by an ROS-independent mechanism [49, 50].

5.3. Cryptococcus neoformans

In 2015, Rocha et al. described that the opportunistic fungus *Cryptococcus neoformans*, which possesses a glucuronoxylomannan (GMX)-containing capside, precludes this fungus to be phagocytosed by neutrophils. These authors also demonstrated that the acapsular strain of *Cryptococcus neoformans*, which harbor glucuronoxylomannogalactan (GMXgal), is capable of inducing NETs. In contrast, the capsular strain does not induce the release of NETs [51].

The release of NETs by the acapsular strain of *Cryptococcus neoformans* is dependent on ROS generation and the PAD4 enzyme. The capsular strain also inhibits PMA-induced NETs formation [51]. NETs release has also been observed in response to *Cryptococcus gattii* stimulation.

Analysis of *Cryptococcus neoformans* susceptibility to acapsular strain-induced NETs showed that NETs diminished colony-forming units (CFUs) by 80% in the capsular strain and by 54% in the case of the acapsular strain. For this, it is necessary that NETs contain MPO.

Paracoccidioides brasiliensis and *Paracoccidioides lutzii* are fungi of the *Paracoccidioides* genus that cause high mortality and morbidity by the systemic mycosis Paracoccidioidomycosis (PCM), mostly in Latin American countries. Della Coletta et al. [52] have investigated the role of neutrophil extracellular traps on these fungi, reporting the formation of NETs by the yeasts *P. brasiliensis* and *P. luttzii*.

6. Neutrophil extracellular traps in viral infections

Viruses have an extraordinary ability to evade the immune system, and the innate immune system is regarded as the first line of defense. Innate immune cells recognize a wide variety of pathogens through their pattern-recognition receptors (PRRs) that include Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-like receptors (RLRs) that recognize pathogen-associated molecular patterns (PAMPs). Several PRRs recognize viral ligands such as TLR-3, TLR-7, TLR-8, RIG-1, and MDA5, and the activation of these PRRs induces the synthesis of antiviral interferons (types I and II), tumor necrosis factor α, interleukin-15, and interleukin-18 [53–55].

The role of NETs in the control of several bacterial infections has been broadly analyzed. However, research on their role in viral infections remains scarce. It has recently been shown that viral infections or virus-derived molecules may act as strong inducers of NETs. Several viruses that induce the formation of NETs have been identified. In some cases, NETs neutralize the viral particles by the MPO or the granule-derived defensins, associated to NETs. The α-defensin protein directly inhibits the influenza virus replication and protein synthesis [56].

Some viruses, such as those of the herpesvirus family, contain proteins with endonuclease activity, so they can degrade NETs and allow viral escape and dissemination. NETs antiviral activity consists in the sequestering of viral particles, thus preventing fusion of viruses with target cells and direct neutralization of virions. It is worth mentioning that viruses do not necessarily infect the neutrophils. However, neutrophils can sense viral particles through their PRRs or via secondary signals produced upon infection of other host cells. The use of secondary signals to induce the release of NETs has important advantages in the context of viral infections [56, 57].

Viruses that induce the release of NETs *in vitro* do so under a non-productive infection of neutrophils. In the case of HIV-1, neutrophils sense this virus by endosomal PRRs that detect viral nucleic acid via TLR-7 and TLR-8, and then undergo NETosis. The respiratory syncytial virus (RSV) induces NETosis through TLR-4. Hantaviruses induce NETs formation by signaling through β_2 integrins. Influenza virus A can stimulate neutrophils directly to release NETs. Viruses also produce NETs indirectly without engagement of the PRRs expressed by neutrophils. Interleukin-8 (IL-8) triggers NETosis. Although NETs formation by viruses is now well established, it is not so clear how NETs contribute to antiviral immunity. In some viruses, as in a mouse model of poxvirus infection, induction of NETs with LPS prior to infection strongly reduced the number of virus-infected liver cells, and this protective effect was reversed by DNAse treatment. Noroviruses can be reduced by their binding to histone H1. Some viral mechanisms counteract NETs formation, as for HIV-1 envelope glycoprotein which stimulates DCs to produce cellular IL-10 through dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN), IL-10 is an immunosuppressive cytokine that, among other functions, inhibits TLR-induced ROS production (54). IL-10 homologs have been found in the genome of large DNA viruses that include ubiquitous human virus, such as human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV). Kaposi's sarcoma-associated herpesvirus (KSHV) impairs the release of NETs, and dengue virus serotype-2 can arrest NETs release by interfering with glucose uptake [6]. Taken together, these findings suggest that virus-induced release of NETs may help to control viral dissemination by direct and indirect mechanisms, whereas, at the same time, viral evasion mechanisms target the formation of NETs.

In 2015, Moreno-Altamirano et al. [16] demonstrated that dengue virus serotype-2 inhibits PMA-induced formation of NETs, arresting neutrophils at the chromatin de-condensation step which, based on a previous report [6], suggests that DENV-2 inhibits the formation of NETs by interfering with glucose uptake and glycolysis.

7. Conclusion

Anti-microbial properties of NETs have been shown for bacteria, protozoa, fungus, and virus. Understanding how neutrophil extracellular traps (NETs) limit the growth of some infectious agents, whereas, apparently, they have no effect on others, and how NETs may cause tissue damage and contribute to the development of pathologies, such as autoimmune diseases, will help to exploit their anti-pathogen properties at full, and to limit their pathogenic effects, in clinical settings. It is quite likely that this research field will continue providing exciting findings.

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