Chapter 1

Neutrophil Extracellular Traps in Infectious Human Diseases

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Additional information is available at the end of the chapter

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Abstract

Neutrophils, as the main cells of the first line of host defense against microbial pathogens, are responsible for pathogen recognition, inhibition of pathogen spreading into the host tissue, and finally, killing the invader cells. Neutrophils carry out these functions via numerous mechanisms, including a relatively recently described activity based on a release of neutrophil extracellular traps (NETs), a process called netosis. NETs are structures composed of DNA backbone, decorated with antimicrobial factors, derived from neutrophil granules. The structure of NETs and their enzymatic and microbicidal inclusions enable efficient trapping and killing of microorganisms within the neutrophil extracellular space. However, the efficiency of NETs depends on neutrophil ability to recognize pathogen signals and to trigger rapid responses. In this chapter, we focus on possible pathways involved in the release of NETs and summarize the current knowledge on triggers of this process during bacterial, fungal, protozoan, and viral infections. We also consider the mechanisms used by microorganisms to evade NET-killing activity and analyze the harmful potential of NETs against the host cells and the contribution of NETs to noninfectious human diseases.

Keywords: neutrophil extracellular traps, netosis, receptors, microbial evasion of NETs, autoimmune diseases

1. Introduction

The human organism is constantly exposed to many microbes, most of them being pathogenic microorganisms that can cause life-threatening infections. The host tissues are a good target for colonization and growth of pathogens; however, the immune system developed during the course of evolution, specialized and responsible for protecting against pathogens, effectively

prevents infections. Among cells of the immune system, polymorphonuclear cells—neutrophils—deserve a special attention. These cells form the first line of defense against pathogens and their components effectively combat the intruders [1]. Neutrophils are phagocytic cells capable of active migration from blood vessels to the site of infection. Their high efficiency in pathogen killing is possible due to a number of factors with microbicidal activity [2]. The main task of neutrophils is capturing pathogens, i.e., reducing the area of infection and inflammation by effective elimination of microorganisms. To fulfill this task, neutrophils use a number of mechanisms. The best-known one is the phagocytosis that involves capturing pathogenic cells, their internalization and killing in special compartments of neutrophil cells—phagosomes [3]. This mechanism, despite its high efficacy and minimal side-effects for the host, can be insufficient to combat massive bacterial infections or attack of other large-size pathogenic cells. An alternative to phagocytosis is a mechanism described in 2004 by Brinkmann et al., involving web-like structures released into the extracellular space, called neutrophil extracellular traps (NETs) [4]. Morphological changes of neutrophils associated with NET formation ("netosis") involve a number of complex intracellular events. The initial process is a decondensation of nuclear chromatin, released into the extracellular space and forming a backbone of vast NETs. These DNA fibers are decorated with associated nuclear proteins—histones—and proteins released from neutrophil granules such as elastase, myeloperoxidase, lactoferrin, and azurocidin [5, 6].

The netosis is classified as a unique type of cell death, different from apoptosis and necrosis. The mechanism of this process is complex and still incompletely understood although the main processes involved have been identified [7, 8]. NETs can be released in response to many different stimuli, including selected chemical compounds, components of pathogen cells, and whole bacteria, fungi, viruses, and parasites [9]. Released structures are able to capture all of these factors and, in consequence, to reduce the pathogen spreading over the host organism. The NET proteinaceous components, often enzymes, are responsible for killing trapped microorganisms, thus restoring the proper functioning of the host body [10]. However, the same components may also destroy surrounding host cells and tissues or trigger some autoimmune diseases [11].

2. Mechanisms of NET formation

The activation of netosis causes dramatic changes in neutrophil morphology involving the decondensation of chromatin, lysis of granules, and cell membrane rupture and leading to neutrophil death called "programmed suicide" which is a third type of neutrophil defensive action, besides phagocytosis and degranulation [4, 6]. However, the newest studies have shown that in some cases neutrophils use exocytosis to release a part of DNA without any rupture of cell membrane, in a process called "vital netosis." However, this term is still under debate because it is not clear, if neutrophils actually remain alive thereafter [12, 13]. Some reports have suggested that in this fast NET-releasing process it is rather the mitochondrial DNA that is excreted, supporting observations of significantly lower efficiency of NET production in comparison with regular netosis [13]. The classical NET-forming pathway is triggered with massive generation of reactive oxygen species (ROS), resulting from the activity of NADPH

oxidase. This ROS-dependent netosis pathway lasts for up to 4 hours, starting from neutrophil activation, and leading to the release of whole nuclear DNA mixed with granular proteins. In contrast, the fast netosis pathway does not require ROS production, leading to a rapid release of NETs within minutes after activation [12].

2.1. Factors that trigger NET production

Netosis can be activated by many compounds, mostly those exposed on the pathogen cell surface. This initial step of NET formation determines the form of released NETs and pathways involved, as well as the intensity and time span of neutrophil response.

The largest group of NET activators are pathogenic Gram-positive and Gram-negative bacteria, but also some fungi (*Aspergillus* spp., *Candida* spp.), as well as viruses (HIV-1, Hantaan virus) and parasites such as *Toxoplasma gondii* and *Leishmania.* Besides microorganisms, numerous chemical factors, including phorbol ester (PMA), hydrogen peroxide, nitric oxide, ionomycin, calcium ions, glucans, mannans, and lipopolysaccharide (LPS), as well as mediators of inflammation such as granulocyte-macrophage colony-stimulating factor (GM-CSF), some interleukins and immune complexes have been identified as potential netosis-triggering factors [9, 11]. Most of them are recognized by neutrophil surface receptors (pattern recognizing receptors, PRRs) that trigger cell signaling for cytokine or chemokine production in order to launch a pathogen-tailored response [14]. Diverse pathogens may be recognized by neutrophils with very similar and overlapping mechanisms.

2.2. Receptors that mediate NET formation

2.2.1. Toll-like receptors

The main PRRs involved in the recognition of pathogens and pathogen-associated molecules are Toll-like receptors (TLRs). Among several TLRs, only TLR2, TLR4, TLR7, and TLR8 have been identified as participating in NET-dependent phenomena. The role of TLR4 in the activation of netosis was confirmed in *Staphylococcus aureus* infection. This receptor plays a great role in the activation of "vital netosis" *in vivo*, cooperating with complement receptor 3 (CR3) [15]. During bacterial sepsis, neutrophils and platelets cooperate in pathogenesis, but the mutual relationship between these cells is still under debate. TLR4, a lipopolysaccharide receptor, seems to mediate the activation of neutrophils by platelets induced by LPS [16].

The other molecule involved in NET triggering via TLRs is high-mobility group box 1 protein (HMGB1). This protein released from dying cells or activated macrophages enhances inflammatory reactions. HMGB1 is a TLR4 agonist, but does not induce the production of ROS by NADPH oxidase, suggesting its involvement in an ROS-independent mechanism of NET formation [17]. On the other hand, an oxidized low density lipoprotein (oxLDL) is able to induce netosis via ROS-dependent pathway, activated by TLR4 and TLR6 receptors [18]. TLR4 was also identified as an important surface recognizing molecule in viruses-activated netosis detected in the lungs of infected hosts. Respiratory syncytial virus (RSV) is responsible for acute bronchiolitis in children under 3 years. This RNA virus exposes a fusion protein (F-protein) on its surface that mediates a fusion of viral envelope with the target cell membrane and also activates NET

release using TLR4 mediation [19]. Moreover, F-protein is also recognized by CD14 receptor, which cooperates with TLR4 [20, 21]. A human immunodeficiency virus HIV-1 is captured and killed in NETs formed by neutrophils using TLR7 and TLR8 to recognize viral nucleic acids. Activation of these receptors leads to production of ROS and activation of ROS-dependent netosis pathway [22].

2.2.2. Receptors of complement system

The most commonly identified receptor of complement system that contributes to neutrophil responses is CR3 complex (Mac-1; CD11b/CD18). It has been identified to be involved in NET triggering by different types of pathogenic microorganisms. The role of Mac-1 in NET formation is best known in fungal life-threatening, systemic infections, especially those caused by *Candida albicans*. On the cell wall, *C. albicans* exposes well-characterized compounds, such as β-glucans or mannans, important for activation of netosis [23–25]. The β-glucan particles are bound by Mac-1 allowing to recognize *C. albicans* at early stage of infection, without preliminary opsonization [26]. Some studies have suggested that for *in vitro* activation of netosis by fungal compounds the presence of fibronectin is required [27]. The activation of Mac-1 causes a rapid formation of NETs via the ROS-independent pathway [26, 27]. However, glucans are also able to induce ROS formation through the activation of NADPH oxidase [28].

Mannheimia haemolytica is a bacterium that causes a severe respiratory disease. One of the virulence factors of this pathogen is leukotoxin (LKT), which can lead to the death of many host cells. LKT was also identified as a *M. haemolytica* factor that triggers NET formation via CD18 receptor, but the complete model of this interaction and the regulation of netosis by this toxin are still not fully understood [29].

Aggregatibacter actinomycetemcomitans, as well as *Actinomyces viscosus* and *S. aureus,* also induce NET release by human neutrophils. However, analysis of the complement receptors involved in netosis activated by these bacteria showed that complement receptor 1 (CR1; CD35) rather than CR3 takes part in recognizing the pathogens [30]. However, CR3 seems to be important for the activation of "vital netosis" induced by *S. aureus* [15].

Moreover, some viruses seem to be recognized by neutrophils via complement receptors. Hantaan virus (HTNV), a member of hantaviruses family, causes severe renal and pulmonary pathologies in humans. This virus is known as a potential NET triggering factor that stimulates neutrophils much stronger than Vaccinia virus or LPS. Detailed analysis of mechanisms of neutrophil activation by HTNV indicated that CR3 and CR4 receptors are necessary for activation of netosis using the ROS-dependent pathway [31].

Another microorganism able to induce netosis is a parasite *Eimeria bovis*. Although this pathogen does not cause diseases in humans but causes diseases in animals, e.g., a severe hemorrhagic diarrhea, especially in calves, it is a good example of activation of netosis via CR3 by parasites. The interaction of Mac-1 with *E. bovis* causes a rapid

Ca^{2+}-mobilization and activation of the ROS-dependent netosis pathway with intensive NET expulsion [32].

Complement receptors are also involved in triggering netosis by immune complexes (ICs) that play an important role in many pathogen-associated diseases, as well as noninfectious, autoimmunological diseases. ICs are bound to neutrophil surface by many different receptors, causing activation of the cells. Mac-1 takes part in these interactions leading to NET release. The overall mechanism is still unclear, but it has been confirmed that IC activation of CR3 receptors leads to the increase of NADPH oxidase activity and, thus, to the initiation of ROS-dependent netosis pathway [33].

2.2.3. Fc-receptors

The recognition of opsonized pathogens or antibody-associated foreign molecules is one of key functionalities of the cells of immune system. In the activation of these cells, antibody receptors of the Fc-receptor family are involved. Neutrophil cells express only two types of surface Fc-receptors for IgG molecules, namely, FcγRIIa (CD32a) and FcγRIIIb (CD16b) [34]. Some microorganisms induce NETs only in the presence of autologous serum [15], suggesting a role of Fc-receptors in the activation of netosis, but it has not yet been resolved which receptors, CD32 or CD16, have greater impact. The best-known NET inducers via Fc-receptors are ICs. Some studies showed that FcγRIIa mediates activation of netosis by endocytosis of ICs [35]. However, other authors suggested that FcγRIIa rather promoted phagocytosis and only FcγRIIIb was involved in the induction of netosis [33]. The activation of netosis by CD16 takes about 3 hours with efficient production of ROS, suggesting a similarity to induction of netosis by PMA.

Fc-receptors also seem to participate in NET formation during bacterial infections. The results presented for neutrophils in contact with opsonized *S. aureus* suggest that activation of Fc-receptors modulates netosis [30]. Moreover, coating of bacteria by IgA also enhances NET formation via FcαIR [36].

2.2.4. C-lectin receptors

C-type lectin receptors (CLRs), such as dectin-1, are responsible for recognition of surface exposed β-glucans of pathogens [37, 38]. The role of glucans in activation of netosis as well as the role of dectin-1 receptor in activation of NET formation are still under debate [26]. The involvement of dectin-1 in this process was confirmed for several fungal pathogens, such as *Paracoccidioides brasiliensis* [39]. However, the role of this receptor in the activation of netosis during *C. albicans* infection is still unclear. Some studies seem to support this hypothesis [40], but, on the other hand, Gazendam et al. suggested that unopsonized *C. albicans* cells do not induce netosis via dectin-1 receptor [26]. The role of dectin-1 was also proposed by Li et al. who showed that upon ligand binding a dectin-1 receptor activates Mac-1, and this receptor induces downstream NET formation [41]. Additional evidence presented that dectin-1 may indirectly mediate netosis depending

on microbial size. Neutrophils in contact with *C. albicans* hyphae or *Mycobacterium bovis* aggregates were able to release NETs. It was proposed that phagocytosis of microbes mediated by dectin-1 plays the function of microbial size sensor and prevents netosis by downregulation of elastase translocation from granules to the nucleus [42]. The number of *Candida* cells and the level of infection were also proposed to be factors responsible for NET formation [43].

Interestingly, the regulation of NET excretion by PMA, used in *in vitro* models of netosis, occurs without activation of any receptors, but directly by the action on protein kinase C (PKC) [44], an important signal mediator of ROS-dependent netosis pathway [45].

2.3. Netosis pathways

Because many of receptors exposed on neutrophil surface are involved and cause cross-activation in NET triggering processes [46–49], the complete pathway of netosis is still under debate. However, some key steps as well as mediating compounds were proposed to be involved in NET formation and are summarized below; however, the specific processes may vary depending on the trigger type.

The first important mediators of netosis, identified in fungal infections associated with NET release, seem to be Src family kinases and spleen tyrosine kinase (Syk) [31, 40]. Src cooperates with plasma membrane-associated receptors, such as CD11b, CD16, or dectin-1, and causes an activation of Syk. Further, Syk devolves the activation signal downstream to next mediators—phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), p38 MAPK (mitogen-activated protein kinase), and extracellular signal-regulated kinases (ERK1/2) pathways [33, 50, 51]. Syk is also involved in the activation of protein kinase C (PKC) by PMA [33, 52, 53], without participation of Src, confirming observed bypassing of the receptors by PMA.

Many of the natural NET inducers, activating the receptors mentioned above, lead to the release of calcium ions from endoplasmic reticulum storage into the cytoplasm, increasing PKC activity [54]. PKC is responsible for phosphorylation of $gp91^{phox}$ that can form the functional complex of NADPH oxidase with subsequent ROS generation [55, 56]. ROS are crucial for classical suicidal netosis (ROS-dependent pathway).

Netosis is a different type of neutrophil death in comparison to apoptosis. Although both mechanisms are mutually exclusive, they could be activated by the same receptors. Indeed, neutrophils are able to block apoptosis, to allow for the formation of NETs. A key molecular switch between apoptosis and netosis seems to be protein kinase B. Activation of Akt allows to induce netosis, but inhibition of this enzyme leads to apoptotic cell death. A key role in apoptosis is played by caspases, whose activities are inhibited in netosis [57]. Moreover, ROS may alternatively inactivate caspases favoring autophagy [58].

The role of PI3K in NET formation is still unclear. Some research showed that phosphorylation of PI3K is not important and has no effect on NET formation via activation of CD16 [59]. On the other hand, an activation of netosis by ICs seems to require active PI3K [33]. Moreover, PI3K

interplays with Akt [60], as well as influences a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) regulation by production of phosphatidylinositol (3,4,5)-trisphosphate [61]. NF-κB has been identified as a regulatory molecule in netosis [62]. PI3K also regulates the autophagy, an important process in PMA- and oxLDL-induced netosis [18, 58, 63].

The role of ERK1/2 in netosis pathway has also been confirmed [19, 32, 33, 59, 64, 65]. ERK1/2 can be induced by Src/Syk, as well as by TLR receptors via interleukin-1 receptor-associated kinase (IRAK) [66]. These mediators seem to be involved in the ROS-dependent netosis pathway, but the relationship between activation of ERK1/2 and generation of ROS by NADPH oxidase is still unsolved. More probably, ERK1/2 can downstream-activate NADPH oxidase [33, 65] or is itself controlled by ROS [45]. The role of p38 MAPK is also not clear, because some studies showed that inhibition of these kinases has no impact on ROS production and ROS-dependent netosis [33, 67, 68], but other presented an opposite effect [32]. The summary of netosis pathways is schematically presented in **Figure 1**.

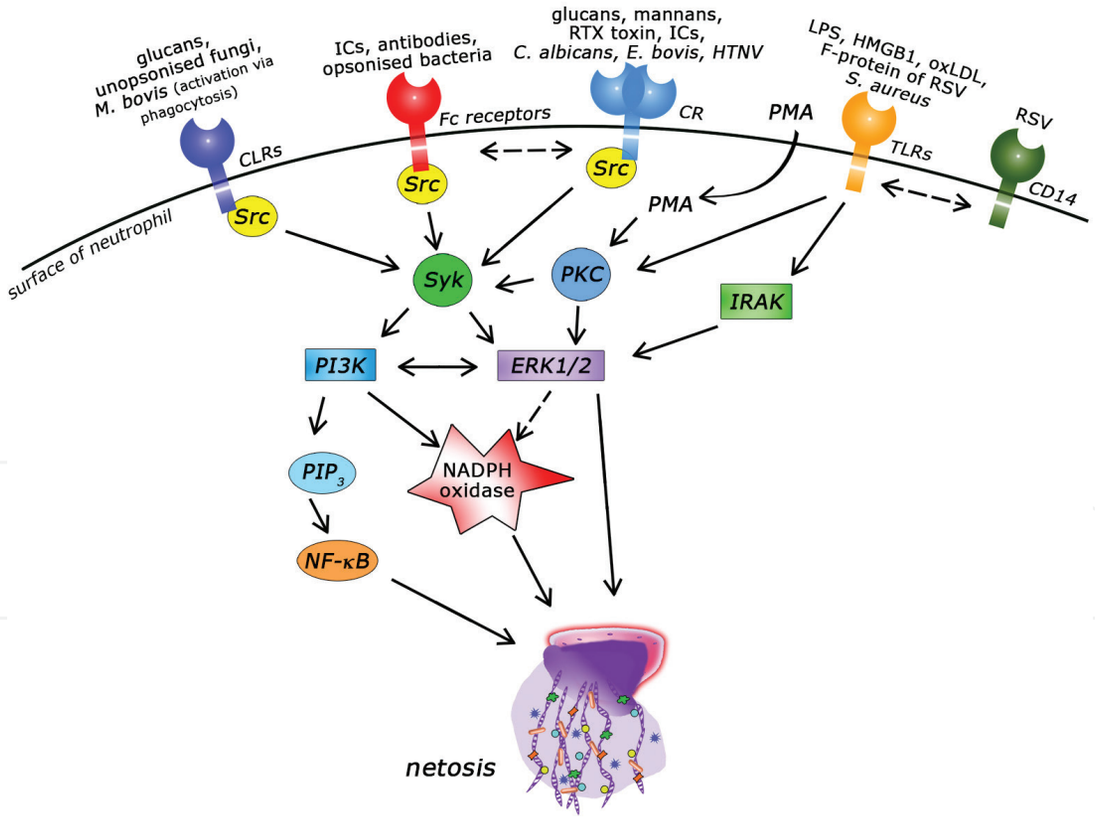

Figure 1. Molecular mechanisms of NET formation. CLRs, C-type lectin receptors; CR, complement receptors; ERK1/2, extracellular signal-regulated kinases; HTNV, Hantaan virus; ICS, immune complexes; IRAK, interleukin-1 receptor-associated kinase; LPS, lipopolysaccharide; PI3K, phosphoinositide 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PMA, phorbol myristate acetate; RSV, respiratory syncytial virus; Src, Src kinase; Syk, spleen tyrosine kinase; TLRs, toll-like receptors.

2.4. Role of ROS in netosis

The first described, classical mechanism of netosis assumed that ROS species play an essential role in netosis (the ROS-dependent pathway) [56]. Indeed, several findings have proven that ROS are key netosis mediators. Patients with chronic granulomatous disease (CGD), caused by a point mutation in gp91^{-phox} subunit of NADPH oxidase, making the enzyme nonfunctional, were more susceptible to infections. Additionally, CGD patients experienced hyper-inflammatory states and sterile inflammations [69, 70]. Moreover, providing ROS from external sources, as well as application to CGD patients a gene therapy, restored the ability of neutrophils to release NETs [8, 46, 71]. Similarly, inhibition of NADPH oxidase by diphenyliodide (DPI) turns off the ability to release NETs [72].

2.5. ROS-independent mechanism of netosis

Little is known about the ROS-independent netosis pathway. NET release without ROS contribution is much faster than the classical netosis. The pathway in which neutrophils remained structurally intact was named as "vital netosis." It can be induced by the same pathogens as those acting in the ROS-dependent manner, e.g., during *Leishmania* parasite infection [12]. Similarly, the induction of NET release in response to glucans of *C. albicans* usually occurs through the ROS-dependent pathway, but in infants, neutrophils release NETs without ROS involvement [73]. Upon contact with *S. aureus* neutrophils release NETs but the web of DNA is released in the exocytosis pathway, without cell membrane rupture. Moreover, NET production was also observed in patients with inactive NADPH oxidase [74]. It was also documented that this type of netosis exploited a release of mitochondrial DNA and an oxidative activity of mitochondrion [13], as well as a small conductance calcium-activated potassium channel 3 (SK3) [75].

2.6. Morphological changes of neutrophils during NET formation

The process of DNA release in the ROS-dependent pathway takes about 1–4 hours and is quite complex. After NADPH oxidase activation, produced ROS probably influence the stability of granules and nuclear envelope. The proteins stored in neutrophil granules—elastase and myeloperoxidase—are moved to the nucleus but the mechanism of their translocation is unknown. In the nucleus, these enzymes contribute to the degradation of linker histones responsible for maintenance of the nuclear structure [55]. They cooperate with next enzyme transferred into the nucleus—peptidyl arginine deiminase 4 (PAD4)—that catalyzes the citrullination of histones, especially H3 and H4. The modification and cleavage of histones lead to the relaxation and decondensation of chromatin, changing the shape and structure of nucleus, and finally causing the disappearance of nuclear membrane [76–78]. DNA is moved into the cytoplasm and mixed with granular proteins such as cathepsin G, proteinase 3, lactoferrin, azurocidin, or with cytoplasmic proteins such as calprotectin [79]. Some research suggests that cytoskeleton also plays an important role in the process of NET formation [46]. At the end of the process, this mixture is released outside the cell. **Figure 2** summarizes all morphological changes during netosis.

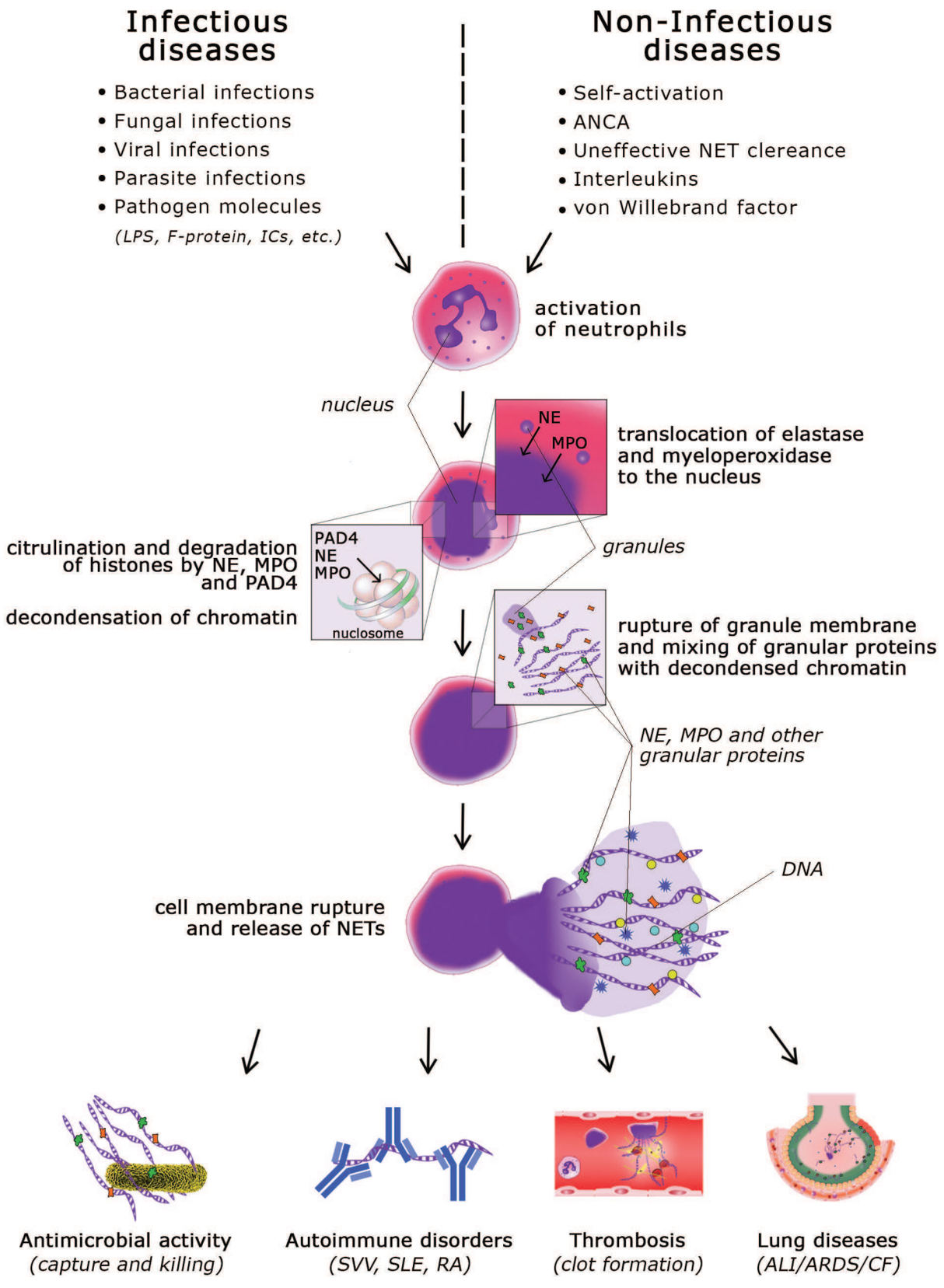

Figure 2. Mechanism of NET formation. ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ANCA, antineutrophil cytoplasmic antibodies; MPO, myeloperoxidase; NE, neutrophil elastase; PAD4, protein arginine deiminase 4; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SVV, small vessel vasculitis.

3. Role of NETs in health and diseases

3.1. The microbicidal activity of NETs

The primary role of NETs is the antimicrobial activity, due to the cooperation of several mechanisms and components exposed at the high local concentrations in the NET fibers [55]. The pathogen spreading is limited by entrapment inside NET structure due to electrostatic interactions between the negatively charged DNA backbone and positively charged bacterial compounds localized on their cell surface [6]. Proteinaceous components of NETs are responsible for different types of NET antimicrobial activities. Proteases such as elastase, cathepsin G, and proteinase 3 are able to cleave virulence factors of *Yersinia enterocolitica*, *Shigella flexneri*, *Salmonella Typhimurium*, and other pathogens [4, 80]. The oxidative mechanisms of defense, e.g., the production of aggressive hypochlorous acid by myeloperoxidase, cause massive damages of NET-entrapped pathogens with their membrane and protein oxidation [81, 82]. Histones, as well as antimicrobial peptides such as LL-37 and BPI, also play an important role in pathogen elimination. Peptides derived from histones and LL-37 take part in cell membrane permeabilization or bacterial cell lysis [83–85]. Moreover, NET-associated factors can restrict nutrient supply for microbes, e.g., lactoferrin chelates iron and calprotectin sequesters zinc ions [79, 84].

3.2. Pathogen escape from NETs

Microorganisms that constantly compete with the host defense mechanisms for survival, elaborated also evasion strategies against toxic effects of NETs. The strategies can be divided into three groups, including: (1) an inactivation of NET components responsible for trapping and killing pathogens, (2) a suppression of NET formation and (3) development of resistance mechanisms against antimicrobial components of NETs.

The main NET component, DNA backbone is degraded by bacterial endonucleases, membrane-bound or released into the surrounding milieu. The group of microorganisms that produce such enzymes to avoid the killing activity of NETs includes *S. aureus* whose nuclease influences the bacterial survival and enhances its infectivity in a mouse respiratory tract infection model [86]. The same strategy, leading to decline NET integrity, is also adopted by other bacteria such as *Aeromonas hydrophila* [87], *Escherichia coli* [88], *Leptospira* sp. [89], *Neisseria gonorhoeae* [90], *Streptococcus agalactiae* [91], *Streptococcus pyogenes* [92, 93], *Streptococcus synguinis* [94], *Streptococcus suis* [95], *Vibrio cholerae* [96], and *Yersinia enterocolitica* [88]. *Streptococcus pneumoniae* uses cell-associated endonuclease (EndA) to escape from local entrapment and promote bacterial spreading from lower airways to bloodstream during pneumonia [97]. Also, parasites such as *Leishmania infantum* use nuclease activity to resist the NET activity [98].

Moreover, the production of ROS involved in the initiation and progression of the main netosis pathway can be regulated by bacterial catalase activity in a self-protection process [99].

Other interesting NET evasion strategies were proposed for meningococci [100], which apply the release of outer membrane vesicles for protection of bacteria from binding to NETs and express a high-affinity zinc uptake receptor (ZnuD) to overcome possible ion sequestration

by calprotectin, the NET component also known to be involved in *C. albicans* killing during netosis [101]. Moreover, a modification of meningococcal LPS with phosphoethanolamine protects bacteria from bactericidal activity of cathepsin G embedded into NET structures.

The bactericidal activity of another NET component, cathelicidin LL-37, can be abolished by its binding to the surface-expressed M1 protein in *S. pyogenes* [102] or to surface exposed D-alanylated lipoteichoic acid in *S. pyogenes* and *S. pneumoniae*, promoting bacteria survival within NETs [103, 104].

Moreover, *C. albicans* aspartic proteases, secreted during NET formation in response to fungal infection, are able to degrade and inactivate LL-37 [105].

Many bacterial toxins are involved in induction of NETs but some of them are used by bacteria to regulate, in particular to inhibit NET formation [106]. *Bordetella pertussis* causing coughing syndrome adopts adenylate cyclase toxin (ACT) to suppress NET shaping [107]. ACT, after translocation into the host phagocyte, may influence the conversion of ATP to cyclic AMP, that in consequence prolongs neutrophil life span by inhibiting the oxidative burst, being one of the initial signals in NET production. This part of NET formation mechanism is also blocked by streptolysin O (SLO) produced by *S. pyogenes* [108].

In the defense against NET formation, microorganisms can also exploit host signaling as in the case of interleukine-8 (IL-8) production by epithelial cells in response to infection. This chemokine is responsible for neutrophil recruiting and amplification of NET release but *S. pyogenes* can produce a peptidase (SpyCEP) which inactivates IL-8 and reduces NET formation [109].

A more complex strategy, used by *Pseudomonas aeruginosa* [110] or *S. agalactiae* [111], employs molecular mimicry with the acquisition of sialic acid motifs presented on the host cell surface which attenuate NET formation. A comparable, indirect mechanism suppressing NET release has been adopted by *Mycobacterium tuberculosis*. This microorganism that triggers NET release during the first stage of infection activates the production of anti-inflammatory cytokine IL-10 that inhibits TLR-induced ROS production and suppresses further NET generation [112].

Also, viruses can apply this strategy of NET suppression, as demonstrated for HIV-1 envelope glycoprotein [22]. Moreover, Dengue virus serotype-2 can negatively affect NET formation by inhibiting glucose uptake in the ROS-independent mechanism of netosis [113].

On the other hand, conidia *of Aspergillus fumigatus* expose hydrophobin (RodA) that suppresses the formation of NETs [114]. This process is also supported by the production of a positively charged exopolysaccharide—galactosaminogalactan that protects the microorganism from binding by NET components [115]. The polysaccharide capsule negatively modulating NET production that contributes to fungal disease severity was also observed in *Cryptococcus neoformans* infections [116].

Another way to subsist the antimicrobial activity of NETs is applied by *P. aeruginosa* in patients with chronic fibrosis where bacteria during its long-term adaptation can form the resistant biofilm that protects the pathogen [117]. Moreover, *S. pneumoniae* and *Haemophilus influenzae* are even able to embed NETs into biofilm for self-protection [118, 119]. Also, the extracellular matrix components of *C. albicans* biofilm alter its recognition by neutrophils and inhibit release of NETs [43].

All the above mechanisms developed by microorganisms to avoid killing by NETs confirm their ongoing adaptation to the sophisticated processes of host defense.

3.3. Role of nets in noninfectious diseases

Netosis is a process being under control of many mechanisms of activation, but NET fibers seem not to be a target or location specific, and in some cases, their release get out of the control. So, the process can be a double-edged sword, acting also against the host cells. Therefore, NETs seem to play a significant role in several autoimmune disease and disorders, described in detail in others reviews [54, 120].

3.3.1. Lung diseases

A chronic inflammatory state of the lungs leads to the development of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) [121–123]. The increased permeability of alveoli due to a mechanical ventilation or infection causes an activation of signaling involved in the release of proinflammatory factors by epithelial cells, and in consequence the massive migration and activation of neutrophils.

NET release can be also the trigger of sterile inflammatory state in the lung. Moreover, a lack of surfactant proteins makes a NET clearance difficult. The proteolytic enzymes contained in NETs damage epithelial cells, in consequence releasing more proinflammatory factors. This generates a self-perpetuating mechanism of netosis activation [11, 124, 125].

A similar mechanism was observed in patients with cystic fibrosis (CF), a disease consisting in an increase in mucus viscosity, therefore hindering the clearance of mucus from the airways [126]. The presence of DNA in CF patient sputum increases a mucus viscosity, which correlates with the development of inflammation state and higher migration of neutrophils. The high viscosity of mucus makes it difficult to remove, generating good conditions for bacterial invasion [126, 127].

3.3.2. Autoimmune disorders

Autoimmune diseases including small vessel vasculitis (SVV), systemic lupus erythematosus (SLE), or rheumatoid arthritis (RA) seem to be also associated with uncontrolled release and ineffective clearance of NETs [128–130]. The high amount of NETs and free-circulating DNA causes a production of antineutrophil cytoplasmic antibodies (ANCAs) against DNA and NET-associated proteins such as MPO, cathepsin G, elastase, etc. Autoantibodies to citrullinated proteins (ACPA) seem to be a key pathologic factor in RA. The circulating complexes of antibodies-DNA or antibodies-NET proteins induce multiorgan inflammatory states, as well as inflammations of vessels [11, 13, 131, 132].

3.3.3. Thromvbosis

Deep vein thrombosis (DVT) is a next pathological state mediated by NETs. Neutrophils can be activated in veins by many different factors, including activated platelets, interleukins, proinflammatory cytokines, as well as von Willebrand factor (vWF), released by NET-damaged endothelial cells. NETs, released inside veins, promote the formation of thrombi by binding

of necessary blood cells and supporting of clot formation. The uncontrolled netosis can lead to massive DVT and consequently to multiple ischemia [11, 13, 133].

4. Conclusions

The progress in investigation of the fundamental processes leading to activation of netosis during pathogenic infection allows us to better understand the main causes of microbial infections and to consider the consequences of neutrophil responses to the host. All of them pointed out on the possible targets for novel therapeutic approaches regulating immunity responses during microbial infection and counteracting the detrimental NET formation and inflammatory diseases.

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