Journal of Applied Biomedicine 16 (2018) 1–9

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

journal homepage: www.elsevier.com/locate/jab

Review article

NETosis – Dr. Jekyll and Mr. Hyde in inflammation

Lucia Lauková*, Barbora Konečná

Comenius University in Bratislava, Faculty of Medicine, Institute of Molecular Biomedicine, Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 18 February 2017
Received in revised form 23 June 2017
Accepted 12 October 2017
Available online 3 November 2017

Keywords:
NETs
NETosis
Extracellular DNA
Inflammation

ABSTRACT

Neutrophils play an important role as the central mediators of the innate immune defence response, providing the first line of host protection. It was shown that these cells can trap and kill various microorganisms through different ways. One of them is a release of neutrophil extracellular traps (NETs) composed of chromatin fibrils and antimicrobial proteins. There is the evidence that the release of NETs does not have only a beneficial effect. NETs can trap and kill microorganisms and pathogens, however on the other hand the same pathway can also cause the damage of the organism by various mechanisms. NETs participate in the pathogenesis of a lot of inflammatory and autoimmune disorders, such as thrombosis, atherosclerosis, cystic fibrosis, periodontitis, lupus, rheumatoid arthritis and others. The aim of this review is to summarize information about the release of NETs and their beneficial, but also detrimental effect during various diseases. The better characterization and understanding of the dual role of NETosis during these diseases is necessary for the early diagnosis and more effective treatment.

Introduction

NETosis is a specific form of cell death performed by neutrophils which is characterized by release of chromatin fibrils into the extracellular space referred to as neutrophil extracellular traps (NETs) (Yang et al., 2016). This process was firstly described by Brinkmann et al. (2004). Since then, mechanism and function of NETosis have been intensively studied.

Neutrophils play a pivotal role in inflammatory response. Variety of neutrophil cell death including apoptosis, necrosis, autophagic cell death and NETosis coexist in response to inflammation. Distribution of cell death phenotypes of neutrophils may be determined by specific factors expressed by individual pathogens, the degree of insult, the duration of stimulation, and the response of the host immune system (Iba et al., 2013). NETosis as well as other types of cell death are mechanisms releasing DNA to the circulation. However, NETosis has several specificities. While apoptotic cells display eat-me signals, such as translocation of phosphatidylserine to the extracellular monolayer of plasma membrane, cells after NETosis do not, which prevents them from clearance by phagocytes. In contrast to apoptosis or necroptosis, granular and nuclear membranes demerge during NETosis, but the integrity of plasma membrane can be retained (Luo and Loison, 2008).

Nowadays, three types of NETosis are known. Suicidal NETosis is the well characterized and most often observed type. This process is oxidant-dependent and includes the cell membrane rupture and the development of cell lysis, which indicates neutrophil death (Kaplan and Radic, 2012). In contrast, in vital NETosis neutrophils release DNA without a loss of nuclear and plasma membrane. That means that after the vital NETosis neutrophils stay alive and are able to phagocyte (Clark et al., 2007). Vital NETosis is divided into the two groups dependently on oxidant formation and origin of the DNA in NETs (Yousefi et al., 2009) (Table 1).

NETs are formed in response to a variety of pro-inflammatory stimuli as well as to several microorganisms in order to trap and kill them (Brinkmann et al., 2004). Today the relationship between NETosis and inflammation is not fully understood. On the one hand, NETosis is the mechanism of immune response and can help to reduce the number of pathogens. On the other hand, NETosis can be connected with inflammatory pathway as an inductor. This dual character can be explained by the fact that NETs are composed of DNA and different types of proteins, which all can induce immune response by their own way (Kaplan and Radic, 2012). The limit, when NETosis is changed from beneficial to detrimental is unknown but NETs showed to be involved in pathogenesis of various inflammatory diseases.

* Author for correspondence: Lucia Lauková, Comenius University in Bratislava, Faculty of Medicine, Institute of Molecular Biomedicine, Sasinkova 4, 811 08 Bratislava, Slovak Republic.
E-mail address: luci.laukova@gmail.com (L. Lauková).

https://doi.org/10.1016/j.jab.2017.10.002
1214-021X/

Table 1
Characterization of different types of NETosis.

Type of NETosis	Suicidal	Vital	
Duration	120–240 min	5–60 min	
Source of DNA	nucleus	nucleus	mitochondria
ROS-dependent	yes	no	yes
Rupture of plasma membrane	yes	no	no
Rupture of nuclear membrane	yes	yes	yes

ROS = reactive oxygen species.

Stimulation and inhibition of NETosis

Formation of NETs represents the response to different pro-inflammatory impulses, such as lipopolysaccharide (LPS), interleukine-8 and tumour necrosis factor α (TNF-α), as well as to various types of pathogens (Table 2). Beside these, NETosis can be stimulated by activated platelets and endothelial cells, nitric oxide, monosodium urate crystals and various autoantibodies or even chemical compounds such as phorbol-12-myristate-13-acetate (PMA) (Kaplan and Radic, 2012).

A physiological inhibitor of NETosis has not been known for a long time. Recent study showed that the blockade of the prostaglandin E2 signalling pathway inhibits NETosis (Domingo-Gonzalez et al., 2016) through the production of cyclic adenosine monophosphate (Shishikura et al., 2016). Modulation of NETosis could be possible also by blocking of inductors or other members of the pathway (Table 2). These findings can contribute to the development of novel treatments for NETosis-related diseases.

NETs structure

Neutrophils have a nucleus consisting of two to five lobes joined together by hair-like filaments. Neutrophils contain granules in cytoplasm, which are classified into three groups based on the presence of proteins. Primary granules comprise mainly the myeloperoxidase (MPO), while secondary granules comprise the lactoferrin, and tertiary gelatinase. They also contain a number of proteins that are important in the initiation of the immune response, such as α-defensins, cathelicidin and others (Borregaard, 2010). NETs are formed by chromatin filaments composed of DNA and histones (Urban et al., 2009). Moreover, the proteins from primary, secondary and tertiary granules of neutrophils are also part of NETs. The granular NET-associated proteins are primarily cationic; therefore, they can effectively bind to DNA molecule. They include: neutrophil elastase (NE), MPO, cathepsin G, proteinase 3, bactericidal/permeability-increasing protein, calgranulin, α-defensins, lactoferrin, a fragment of the protein cathelicidin hCAP18–the peptide LL-37, pentraxin, matrix metalloproteinase-9 (MMP-9) and peptidoglycan recognition protein-S (Table 3) (Averhoff et al., 2008; Bianchi et al., 2011; Parker et al., 2012).

Table 2
Inductors and inhibitors of NETosis.

Inductors	Article
Gram-positive bacteria	Malachowa et al. (2013), Ramos-Kichik et al. (2009)
Gram-negative bacteria	Juneau et al. (2011), Marin-Esteban et al. (2012)
Virus	Narasaraju et al. (2011)
Protozoa	Abi Abdallah et al. (2012), Ventura-Juarez et al. (2016)
Yeasts	Byrd et al. (2013)
PMA	Keshari et al. (2013)
LPS	Brinkmann et al. (2004)
Cytokines	Alfaro et al. (2016), de Boer et al. (2013)
Chemokines	Rossaint et al. (2014)
Immune-complexes	Behnen et al. (2014)
Anti-neutrophil antibodies	Sha et al. (2016)
Activated platelets	Clark et al. (2007)
Uric acid	Arai et al. (2014)
Monosodium urate	Schorn et al. (2012)
Ca^{2+} ionophore	Palić et al. (2007)
Oxidants	Keshari et al. (2013)
Heavy metals	Haase et al. (2016)
Glucose	Miyoshi et al. (2016)
Cholesterol crystals	Neumann et al. (2014a)
LL-37	Tripathi et al. (2014)
High-mobility group Box 1 protein	Tadie et al. (2013)
Complement component 5a	Huang et al. (2015)
Inhibitors	
Deoxyribonucleases	Halverson et al. (2015)
Vitamin C	Mohammed et al. (2013)
Gallic acid	Haute et al. (2015)
Acetylsalicylic acid	Lapponi et al. (2013)
Metformin	Wang et al. (2015)
Antiproteases	Majewski et al. (2016), Zabieglo et al. (2015)
Ascomycin	Gupta et al. (2014)
Cyclosporine	Gupta et al. (2014)
Inhibitors of PAD4	Knight et al. (2015),Kusunoki et al. (2016)
Inhibitors of MPO	Metzler et al. (2011)
Inhibitor of NF-κB	Lapponi et al. (2013)
Antibodies against cytokines	Keshari et al. (2012)
Thrombomodulin	Shimomura et al. (2016)
Activated protein C	Healy et al. (2017)

Note: PMA, phorbol-12-myristate-13-acetate; LPS, lipopolysaccharide; PAD4 ,peptidyl-arginine deiminase 4; MPO, myeloperoxidase; NF-κB, nuclear factor-kappaB.

Table 3
NET-associated proteins and their origin and function.

Protein name	Localization in cell	Function	Publication
Histones	Nucleus	Bactericidal function	Hirsch (1958); Wang et al. (2011)
Myeloperoxidase	Primary granules	Synthesis of HOCl and chromatin decondensation	Papayannopoulos et al. (2010)
Neutrophil elastase	Primary granules	Degradation of the histone H1 and modification of other histones	Korkmaz et al. (2008), Massberg et al. (2010)
Peptidyl-arginine deiminase 4		Citrullination of histones	Li et al. (2010), Neeli et al. (2008)
Cathepsin G	Primary granules	Antimicrobial activity and stimulation of coagulation	Korkmaz et al. (2008), Massberg et al. (2010)
Proteinase 3	Primary granules	Antimicrobial activity	Korkmaz et al. (2008)
α-defensins	Primary granules	Antimicrobial activity	Brook et al. (2016)
Bactericidal/permeability-increasing protein	Primary granules	Bactericidal activity	Skopelja et al. (2016)
Lactoferrin	Secondary granules	Antimicrobial and anti-inflammatory activity	Baker and Baker (2005)
Pentraxin 3	Secondary granules	Antimicrobial activity and control of autoimmunity	Jaillon et al. (2007)
LL-37	Secondary granules	Antimicrobial activity	Neumann et al. (2014b)
Calprotectin	Cytoplasm	Antimicrobial activity and recruitment of inflammatory cells	Urban et al. (2009)
Catalase	Peroxisomes	Catalyzation of decomposition of hydrogen peroxide	Palmer et al. (2012a)

Mechanism of NETs formation in suicidal and vital NETosis

The process of suicidal NETosis, which is the most common type of NETosis, starts with the activation of neutrophils after recognition of an inductor (Figs. 1 and 2). The most effective stimulator of this pathway is PMA; therefore, it is frequently used as an activator of NETosis. Activation of neutrophils leads to the stimulation of protein kinase C activity and this induces formation of NADPH oxidase (NOX) complex on plasma and granule membranes (Fuchs et al., 2007; Guimarães-Costa et al., 2012). Then NOX enzymes ensure the transport of electrons across the plasma membrane and to molecular oxygen, which leads to reactive oxygen species (ROS) production. Under the impact of ROS, plasma and granule membrane break and the content of a nucleus, granules and cytoplasm is mixed (Yang et al., 2016). The next activating step is chromatin decondensation, in which activity of enzymes NE, MPO and peptidyl-arginine deiminase 4 (PAD4) is crucial as proved in murine model (Hemmers et al., 2011; Li et al., 2010; Metzler et al., 2011; Papayannopoulos et al., 2010). NE degrades linker histone H1 and modifies core histones, while MPO intensifies chromatin decondensation by the synthesis of hypochlorous acid (Papayannopoulos et al., 2010). PAD4 deiminases core histone proteins while arginine is converted to citrulline. Citrullination of histones results in their weakened binding to DNA (Neeli et al., 2008). The process of suicidal NETosis is finished after the rupture of plasma membrane and release of NETs into extracellular space.

Contrary to suicidal NETosis, neutrophils stay alive and are able to phagocytose after vital NETosis (Table 1). This process occurs in response to some types of pathogens and is rapid (5–60 min). Vital NETosis consists of a growth of nuclear envelope, chromatin decondensation and disruption of nuclear membrane (Pilsczek et al., 2010; Yipp and Kubes, 2013). However, neutrophils do not lose nuclear and plasma membrane during this type of NETosis. Induction of vital NETosis is through the stimulation of toll-like receptors (TLR) and the complement receptor for C3 protein (Delgado-Rizo et al., 2017). Despite the loss of nuclear DNA, neutrophils maintain their chemotactic ability and are able to chase and phagocyte bacteria.

The special type of vital NETosis is mitochondrial NETosis, which is ROS-dependent contrary to nuclear vital NETosis. In this case NETs are created from mitochondrial DNA (McIlroy et al., 2014). It is still unclear why the neutrophils choose the particular type of NETosis and whether it is dependent on the type or intensity of stimulating factor.

Importance of NETs

Capturing of the pathogens is important for the limitation of their extension from the initial site of infection. The key

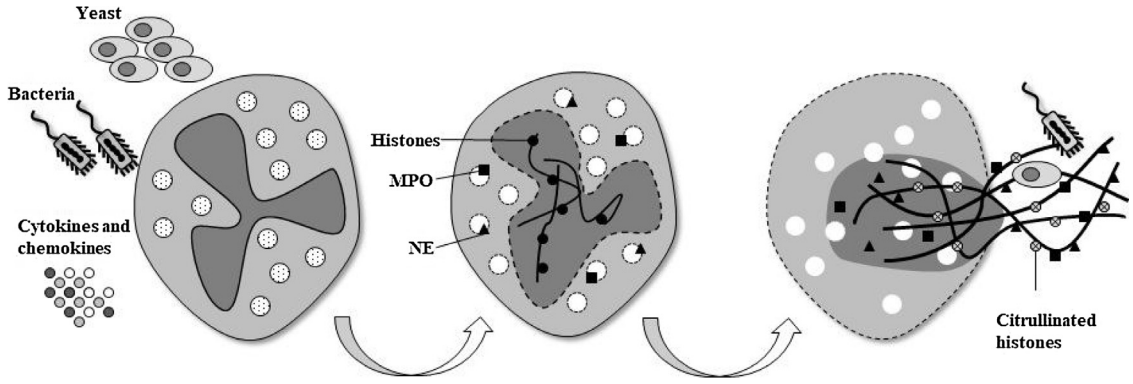

Fig. 1. Schematic representation of NETosis.
Neutrophil activation by biological or chemical stimuli (cytokines, chemokines, bacteria, yeast . . .) is followed by the nuclear chromatin decondensation and the release of enzymes (MPO = myeloperoxidase, NE = neutrophil elastase) from granules. Histone proteins are citrullinated and neutrophil extracellular traps are released to extracellular space where they trap microorganisms.

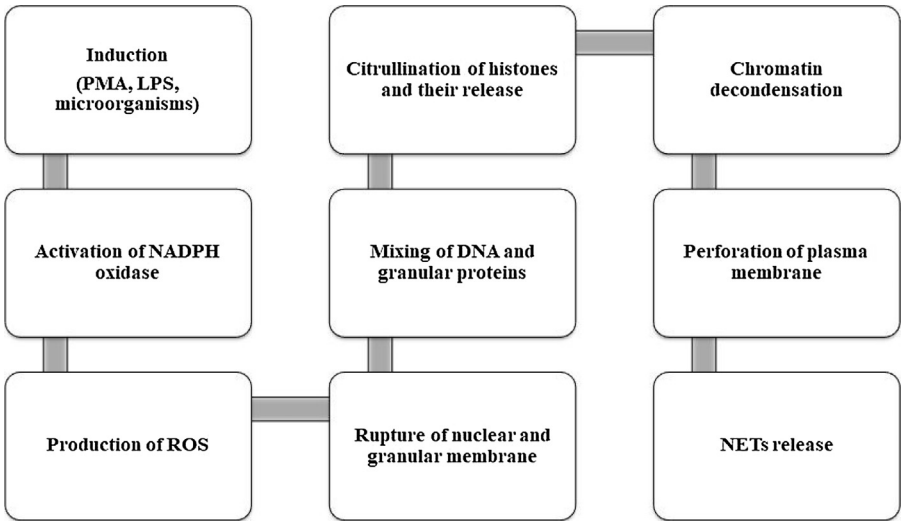

Fig. 2. Mechanism of release of neutrophil extracellular traps (NETs) during suicidal NETosis.
Inductors such as phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharide (LPS) are recognised by receptors and activate NADPH oxidase, which leads to the production of reactive oxygen species (ROS). ROS generation results in the rupture of nuclear and granular membranes and mixing of contents. Citrullination of histones by peptidyl arginine deiminase (PAD4) as well as their degradation with proteases leads to chromatin decondensation and formation of NETs.

mechanism, by which bacteria are trapped by NETs, are the electrostatic interactions between positively charged bacterial membrane and negatively charged DNA of chromatin fibrils (Brinkmann and Zychlinsky, 2007). However, some types of bacteria which have a capsule on their surface or that can change their surface charge, are protected from being caught (Wartha et al., 2007). Another way to avoid entrapping is the production of nucleases and their expression on the surface of the bacteria. This ability to synthesize nucleases protecting them from NETs was demonstrated just in *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pneumococcus* species (Beiter et al., 2006).

The next way, how NETs eliminate the infection is the inactivation of virulence factors that can modify the features of host cells. NE, proteinase 3 and cathepsin G have this ability (Averhoff et al., 2008; Weinrauch et al., 2002). Several components of NETs can stop and kill pathogens in other ways. These include proteases, lysozymes, antimicrobial peptides, ion chelators and histones. The action of these factors and their concentration in chromatin fibrils determine antimicrobial activity of NETs. MPO effectively kills microorganisms during antimicrobial oxidative bursts by converting diffusible long-lived hydrogen peroxide into highly reactive and locally confined hypochlorous acid (Schürmann et al., 2017). Other components of NETs include calgranulin and cathelicidin. Calgranulin as a chelator of zinc has fungicidal activity (Bianchi et al., 2011), while antimicrobial peptide cathelicidin binds to extracellular DNA to keep itself from the endonucleases, thus to prolong NETosis (Neumann et al., 2014b). Histones are bactericidal and antiprotozoal. Indeed, they are also able to kill the cells of a host and are closely related to the pathogenesis of sepsis (Saffarzadeh et al., 2012; Xu et al., 2009) and addition of antibodies against histones result in the inhibition of the killing pathogens (Brinkmann et al., 2004).

Involvement of NETosis in pathophysiologic processes

In contrast to antimicrobial function of NETosis is its involvement in noninfectious, sterile inflammation (Fig. 3). There is evidence that NETosis has an ability to amplify and under specific conditions also overstimulate the immune response. It is unclear whether all NETs are equally harmful for the host and whether NETs from various diseases are phenotypically and functionally identical or not (Kaplan and Radic, 2012).

Alarmins, also called danger-associated molecular patterns (DAMPs), are endogenous molecules recognised by the immune system of a host, able to induce the sterile inflammation. These molecules include heat shock proteins, high-mobility group Box 1 protein, but also extracellular DNA and RNA. Many structural compounds of NETs are alarmins; i.e. DNA as a main component of NETs can be sensed by endosomal TLR9 in plasmacytoid dendritic cells (pDCs) and monocytes, as well as by various cytosolic receptors in macrophages (Lande et al., 2007). Activation of these receptors leads to the synthesis of pro-inflammatory cytokines. The next detrimental side of excessive NETosis is the fact that it leads to tissue damage due to the epithelial and endothelial cell death (Xu et al., 2009). Level of damage and organ dysfunction depends on the intensity of NETs release (Caudrillier et al., 2012; Narasaraju et al., 2011). Therefore, NETosis is involved in the pathogenesis of various disorders including cardiovascular and autoimmune diseases. Below, recent discoveries on the potential role of NETs in the pathogenesis of some of them are summarized.

Cardiovascular diseases

NETosis was observed in association with several cardiovascular diseases, such as thrombosis and atherosclerosis. Thrombosis is a formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. Neutrophils are accumulated and tightly adhere to the affected endothelium, where they produce NETs. NETs work as a scaffold for capturing of platelets and erythrocytes, thus contributing to the thrombus formation. Due to their negative charge, NETs are able to bind plasma proteins such as fibrin and von Willebrand factor and fix the clot (Fuchs et al., 2010). Moreover, NE and cathepsin G are proteases, which cleave a tissue factor pathway inhibitor and also anticoagulants such as antithrombin and activated protein C, thereby stimulate coagulation, as proved in murine model (Massberg et al., 2010). The next way how NETs can contribute to the thrombus formation is the activation of factor XII and factor VII (von Brühl et al., 2012). Histones are also able to activate platelets and the generation of thrombin (Carestia et al., 2013). The manner, in which histones stimulate coagulation, is the interaction with thrombomodulin and protein C, which leads to the inhibition of activation protein C and results in thrombin generation. Role of

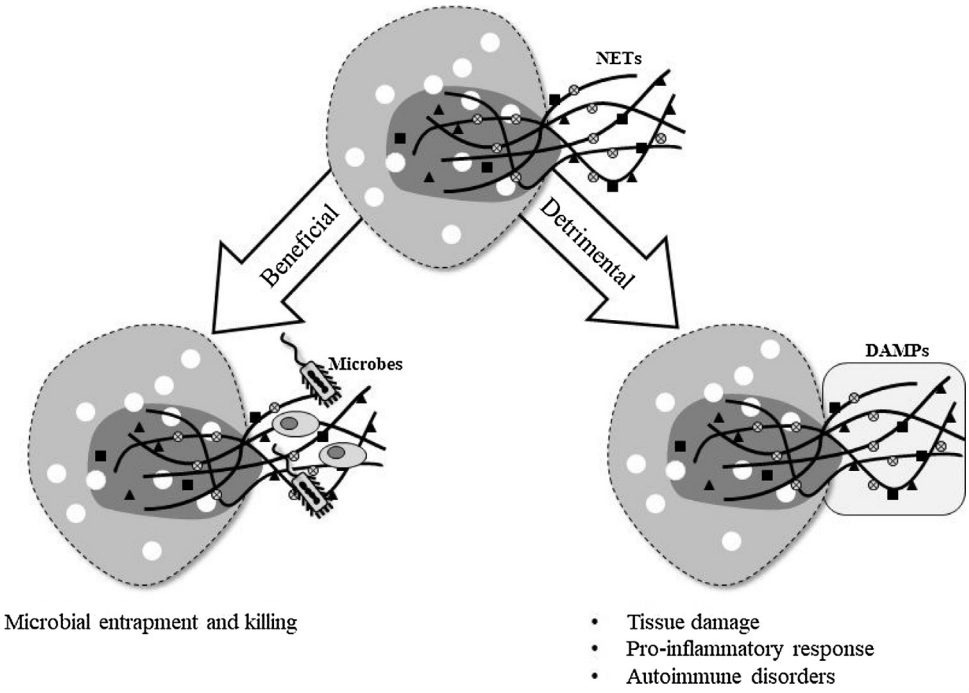

Fig. 3. Advantages and disadvantages of NETosis.
The formation of neutrophil extracellular traps (NETs), induced by pathogens is crucial for microbial trapping and killing. However the excessive generation of NETs results in tissue damage, activation of proinflammatory response and autoimmune disorders. (DAMPs = damage-associated molecular patterns).

NETs and their components in pathophysiology of thrombosis was proved also by deoxyribonuclease (DNase) and the anti-NET antibody treatment, which decreased clot formation in murine model (Brill et al., 2012; Massberg et al., 2010).

Atherosclerosis is a chronic inflammatory disease, in which nodules and plaques build up inside the arteries. Atherosclerotic plaques as well as the damaged vessel walls contain components of NETs, such as peptides LL-37 and its analogue cathelin-related antimicrobial peptide (Cramp) (Döring et al., 2012a, b). Cramp$^{-/-}$ mice fed with high-fat diet exhibit reduced lesion sizes with lower macrophage and neutrophil numbers in comparison to controls (Döring et al., 2012a). Also complexes of citrullinated histones with DNA and MPO/DNA complexes were found in coronary vessel walls in patients with coronary artery disease. These DNA-protein complexes are also present in blood whereby their level increases in direct proportion to the severity of the general atherosclerosis. Moreover, the level of MPO/DNA complexes could serve as a predictive biomarker of the number of coronary vessels involved in atherosclerosis as was proved in the clinical study (Borissoff et al., 2013).

Immunopathogenesis of atherosclerosis is connected with increased level of pDCs in affected vessel walls and occurrence of immunostimulatory DNA-protein complexes. Components of NETs activate pDCs which results in high synthesis of interferons in vessel walls. The removal of pDCs had a positive effect and reduced the formation of atherosclerotic plaques in mice. Activation of pDCs and the production of interferon I resulted in an intensive development of atherosclerotic plaques (Döring et al., 2012b).

Furthermore, NETosis causes damage to the vascular endothelium. The endothelial dysfunction is caused by MMP-9 as the next component of the NETs. This enzyme induces the cell death and causes the dysfunction of endothelium by activation of endothelial MMP-2. The repression of NETosis and MMP-2 leads to the restoration of normal function of endothelium (Carmona-Rivera et al., 2015), therefore the detection of NETs can be a good diagnostic tool.

Autoimmune diseases

Systemic lupus erythematosus (SLE) is a chronic autoimmune systemic disease characterized by the production of autoantibodies, deposition of immune complex in tissues, infiltration of tissues with T and B cells, neutrophils and macrophages, and subsequent inflammatory tissue injury (Liu and Davidson, 2012). Autoantibodies are specifically targeted not only against nuclear antigens such as histones, DNA and ribonucleoproteins, but also against granular proteins of neutrophils typical for NETs. Therefore NETs are considered as a potential source of autoantigens. The deficiency of NET clearance is connected with high titers of anti-NET antibodies and renal involvement in patients with SLE correlates with the lack of NET clearing (Hakkim et al., 2010). Moreover, the concentration of NET-associated proteins, such as defensins, high-mobility group box protein 1, LL-37 and other bactericidal proteins in blood of lupus patients is higher compared to healthy controls. These can then be the good target for autoantibodies and may worsen the disease activity (Ma et al., 2012). The other problem, which can help NETs in exacerbation of the disease, is a deficiency in DNase activity. This lack of DNA cleavage was shown especially in patients with inherited form of SLE, who had a mutation in gene for DNase 1 or DNase1-like 3 (Al-Mayouf et al., 2011; Yasutomo et al., 2001). DNase gene polymorphisms, DNase 1 inhibitors and high amount of anti-NET antibodies were found in SLE patients (Kim et al., 2008; Shin et al., 2004). All these reasons led to the lack of NETs degradation (Hakkim et al., 2010).

As mentioned previously, NETs are recognized by immune system as DAMPs by various receptors, including TLR9, which induces imbalanced immune homeostasis (Kahlenberg et al., 2013). Interestingly, mice lacking functional TLR9 had more serious disease and a shortened lifespan (Nickerson et al., 2013; Stoehr et al., 2011). Moreover, the deficiency of the NOX2, which should inhibit the pathogenesis of the disease in lupus-prone mice by the inhibition of suicidal NETosis, caused exacerbation of SLE

(Campbell et al., 2012). The clarification and characterization of the role of NETs in the pathogenesis of SLE is therefore necessary for the acquisition of new therapeutic approaches.

Rheumatoid arthritis (RA) is an autoimmune disease, which involves mainly the inflammation of joints, but similarly to SLE can also affect internal organs. Pathophysiology of RA as well as SLE is associated with NETs. Serum of RA patients contains a lot of various autoantibodies, the most of them targeted to citrullinated proteins, which are characteristic for NETs (Szekanecz et al., 2008). Neutrophils isolated from blood and synovial fluid of RA patients undergo NETosis more spontaneously compared to healthy controls (Sur Chowdhury et al., 2014). The amount of NETs in blood of RA patients correlated with the presence of anticitrullinated peptide antibodies and cytokine production (Khandpur et al., 2013). Contrarily, the PAD4-deficiency had no effect on the development or severity of the disease in mouse model of RA (Rohrbach et al., 2012). These opposing views on the impact of NETosis in this autoimmune disease can be elucidated just by extensive study and usage of more experimental models.

The next autoimmune disease, in which NETosis could play a role, is type 1 diabetes mellitus (T1DM). T1DM is characterized by the inability of pancreas to produce insulin due to the autoimmune destruction of beta cells resulting in hyperglycemia. Several studies showed that T1DM patients suffer from neutropenia, which may be attributed to infiltration of neutrophils in pancreatic tissue and increased NETosis (Harsunen et al., 2013; Valle et al., 2013). Neutrophils exposed to hyperglycemic conditions are stimulated to produce ROS and cytokines. A concentration of TNF-α is increased in plasma of diabetic patients and this induces NETosis. By this way NE, cathepsin G and proteinase 3 are freed while elevated concentration and activity of these enzymes are shown in T1DM patients (Wang et al., 2014). Moreover, these proteins are able to induce the production of cytokines and expression of TLRs, which are mediators of insulitis and beta cells destruction (Padgett et al., 2013).

Cystic fibrosis

Cystic fibrosis is a hereditary autosomal recessive disorder that causes severe damage to the lungs, digestive system and other organs in the body. Cystic fibrosis affects the cells that produce mucus, sweat and digestive juices and is characterized by the increased number of neutrophils as well as NETs in the lungs (Marcos et al., 2010). High level of extracellular DNA, some of which originates from the NETs, increases sputum viscosity in patients with cystic fibrosis (Yoo et al., 2014). In order to reduce the sputum viscosity, these patients are treated with recombinant DNase I. However, DNase I is not able to effectively cleave the complexes consisting of DNA and histones typical for NETs because a lot of cleavage sites are hidden. The characterization of the chromatin structures in sputum from patients who were not under DNase I therapy showed that previous treatment with NE enhances solubilisation of sputum by cleaving histones, increasing the access of DNases to DNA (Papayannopoulos et al., 2011). The appropriate adjustment of therapy targeted to reduce the NETs in lungs than can be useful for the treatment not just the cystic fibrosis patients but also patients with other diseases associated with sputum accumulation, such as chronic obstructive pulmonary disease (Grabcanovic-Musija et al., 2015).

Periodontitis

Periodontitis is one of the most common chronic inflammatory diseases, which causes serious gum infection that damages the soft tissue and destroys the bone that supports teeth and causes their loss (Farquharson et al., 2012; Kassebaum et al., 2014). The first step of the initiation of the development of periodontitis is a disbalance within the accumulated plaque of biofilm followed by bacterial infections (Bascones-Martínez et al., 2009). Neutrophils are important for bacterial clearance during infection but in sensitive patients, they are not able to eliminate the pathogenic bacteria. Production of cytotoxic components including ROS during NETosis is important to destroy the bacteria, however neutrophils isolated from chronic periodontitis patients are hyper-reactive regarding ROS release, which may lead to the progression of the disease (Matthews et al., 2007).

Long-term periodontitis and hypercoagulability may cause the atherothrombosis (Demmer and Desvarieux, 2006). It is suggested that repeated injections with *Porphyromonas gingivalis*, which is commonly responsible for periodontitis, cause abdominal aortic aneurysms as proved in rat model (Delbosc et al., 2011). In this study, thrombi observed in rats after the infection contained high level of NETs (Delbosc et al., 2011). Determination of the concentration of mediators, which are able to stimulate the production of NETs, such as interferon-α in serum of periodontitis patients showed that NETosis may be an effective biomarker of periodontitis (Wright et al., 2008). On the contrary, reduced amount of NETs in serum of patients with periodontitis may increase their sensitivity to the colonization of bacteria. This effect may be due to production of DNase by bacteria. Palmer et al. suggested that many periodontal bacteria release DNases, which can help them to enter the tissue and prevent their abolishment (Palmer et al., 2012b). The same authors also demonstrated that the activation of neutrophils to release the NETs differed between specific periodontal bacteria *in vivo* (White et al., 2014), which can also help them with persistence of the infection. The role of NETosis in periodontitis is undoubtedly important, but further studies are needed for its explanation and better understanding.

Conclusion

The evidence that NETosis participates on pathophysiology of cardiovascular diseases, autoimmune diseases as well as other pathologies such as cancer and Alzheimer disease increases during last years. Regardless of intensive research and the development of this field of study, there are still lots of questions, which are important for better understanding of the pathophysiological impact of NETosis. It is necessary to clarify the critical boundary when NETosis changes from helpful to destructive as well as to characterize if these neutrophils and their NETs, which participate on diseases, have any composition differences. Understanding precisely if sterile inflammatory NETs differ structurally from NETs released during infection requires the progress in this field. More work in experimental models of diseases as well as prospective studies are also needed to assess if presence of NETs and their components can be used as biomarkers in patients with autoimmune and other diseases. Moreover, the lack of the standardized methods for the detection of NETs and NET-associated proteins is also a problem because there are no commercial kits for reliable detection. The simple visualization and quantification of NETs using DNA-intercalating dyes or immunofluorescence microscopy using antibodies against NET-associated proteins have limitations and it is time-consuming. Thus, there is a requirement for techniques, which are able of robust and rapid evaluation of NET-releasing cells, for example, by flow cytometry combined with imaging. The solving of these problems and ambiguities would be helpful for more accurate diagnosis, prognosis and for the target of the treatment of many diseases.

Conflicts of interests

The authors declare no conflict of interests.

References

Abi Abdallah, D.S., Lin, C., Ball, C.J., King, M.R., Duhamel, G.E., Denkers, E.Y., 2012. Toxoplasma gondii triggers release of human and mouse neutrophil extracellular traps. Infect. Immun. 80, 768–777.

Al-Mayouf, S.M., Sunker, A., Abdwani, R., Abrawi, S., Al Almurshedi, F., Alhashmi, N., et al., 2011. Loss-of-function variant in DNASE1L3 causes a familial form of systemic lupus erythematosus. Nat. Genet. 43, 1186–1188.

Alfaro, C., Teijeira, A., Oñate, C., Pérez, G., Sanmamed, M.F., Andueza, M.P., et al., 2016. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). Clin. Cancer Res. 22, 3924–3936.

Arai, Y., Nishinaka, Y., Arai, T., Morita, M., Mizugishi, K., Adachi, S., et al., 2014. Uric acid induces NADPH oxidase-independent neutrophil extracellular trap formation. Biochem. Biophys. Res. Commun. 443, 556–561.

Averhoff, P., Kolbe, M., Zychlinsky, A., Weinrauch, Y., 2008. Single residue determines the specificity of neutrophil elastase for Shigella virulence factors. J. Mol. Biol. 377, 1053–1066.

Baker, E.N., Baker, H.M., 2005. Molecular structure, binding properties and dynamics of lactoferrin. Cell. Mol. Life Sci. 62, 2531–2539.

Bascones-Martínez, A., Muñoz-Corcuera, M., Noronha, S., Mota, P., Bascones-Ilundain, C., Campo-Trapero, J., 2009. Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. Med. oral, Patol. oral y cirugi'a bucal 14, e680–685.

Behnen, M., Leschczyk, C., Möller, S., Batel, T., Klinger, M., Solbach, W., Laskay, T., 2014. Immobilized immune complexes induce neutrophil extracellular trap release by human neutrophil granulocytes via FcγRIIIB and Mac-1. J. Immunol. 193, 1954–1965.

Beiter, K., Wartha, F., Albiger, B., Normark, S., Zychlinsky, A., Henriques-Normark, B., 2006. An endonuclease allows Streptococcus pneumoniae to escape from neutrophil extracellular traps. Curr. Biol. 16, 401–407.

Bianchi, M., Niemiec, M.J., Siler, U., Urban, C.F., Reichenbach, J., 2011. Restoration of anti-Aspergillus defense by neutrophil extracellular traps in human chronic granulomatous disease after gene therapy is calprotectin-dependent. J. Allergy Clin. Immunol. 127, 1243–1252.

Borissoff, J.I., Joosen, I.A., Versteylen, M.O., Brill, A., Fuchs, T.A., Savchenko, A.S., et al., 2013. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. Arterioscler. Thromb. Vasc. Biol. 33, 2032–2040.

Borregaard, N., 2010. Neutrophils, from marrow to microbes. Immunity 33, 657–670.

Brill, A., Fuchs, T.A., Savchenko, A.S., Thomas, G.M., Martinod, K., De Meyer, S.F., et al., 2012. Neutrophil extracellular traps promote deep vein thrombosis in mice. J. Thromb. Haemost. 10, 136–144.

Brinkmann, V., Zychlinsky, A., 2007. Beneficial suicide: why neutrophils die to make NETs. Nat. Rev. Microbiol. 5, 577–582.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., et al., 2004. Neutrophil extracellular traps kill bacteria. Science 303, 1532–1535.

Brook, M., Tomlinson, G.H., Miles, K., Smith, R.W.P., Rossi, A.G., Hiemstra, P.S., et al., 2016. Neutrophil-derived alpha defensins control inflammation by inhibiting macrophage mRNA translation. Proc. Natl. Acad. Sci. 113, 4350–4355.

Byrd, A.S., O'Brien, X.M., Johnson, C.M., Lavigne, L.M., Reichner, J.S., 2013. An extracellular matrix-based mechanism of rapid neutrophil extracellular trap formation in response to Candida albicans. J. Immunol. 190, 4136–4148.

Campbell, A.M., Kashgarian, M., Shlomchik, M.J., 2012. NADPH oxidase inhibits the pathogenesis of systemic *Lupus erythematosus*. Sci. Transl. Med. 4, 157ra141.

Carestia, A., Rivadeneyra, L., Romaniuk, M.A., Fondevila, C., Negrotto, S., Schattner, M., 2013. Functional responses and molecular mechanisms involved in histone-mediated platelet activation. Thromb. Haemost. 110, 1035–1045.

Carmona-Rivera, C., Zhao, W., Yalavarthi, S., Kaplan, M.J., 2015. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. Ann. Rheum. Dis. 74, 1417–1424.

Caudrillier, A., Kessenbrock, K., Gilliss, B.M., Nguyen, J.X., Marques, M.B., Monestier, M., et al., 2012. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J. Clin. Invest. 122, 2661–2671.

Clark, S.R., Ma, A.C., Tavener, S.A., McDonald, B., Goodarzi, Z., Kelly, M.M., et al., 2007. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat. Med. 13, 463–469.

Döring, Y., Drechsler, M., Wantha, S., Kemmerich, K., Lievens, D., Vijayan, S., et al., 2012a. Lack of neutrophil-derived CRAMP reduces atherosclerosis in mice. Circ. Res. 110, 1052–1056.

Döring, Y., Manthey, H.D., Drechsler, M., Lievens, D., Megens, R.T.A., Soehnlein, O., et al., 2012b. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. Circulation 125, 1673–1683.

de Boer, O.J., Li, X., Teeling, P., Mackaay, C., Ploegmakers, H.J., van der Loos, C.M., et al., 2013. Neutrophils, neutrophil extracellular traps and interleukin-17 associate with the organisation of thrombi in acute myocardial infarction. Thromb. Haemost. 109, 290–297.

Delbosc, S., Alsac, J.M., Journe, C., Louedec, L., Castier, Y., Bonnaure-Mallet, M., et al., 2011. Porphyromonas gingivalis participates in pathogenesis of human abdominal aortic aneurysm by neutrophil activation. Proof of concept in rats. PLoS One 6, e18679.

Delgado-Rizo, V., Martínez-Guzmán, M.A., Iñiguez-Gutierrez, L., Garcia-Orozco, A., Alvarado-Navarro, A., Fafutis-Morris, M., 2017. Neutrophil extracellular traps and its implications in inflammation: an overview. Front. Immunol. 8, 81.

Demmer, R.T., Desvarieux, M., 2006. Periodontal infections and cardiovascular disease: the heart of the matter. J. Am. Dent. Assoc. 137 (Suppl), 14S–20S (quiz 38S).

Domingo-Gonzalez, R., Martínez-Colón, G.J., Smith, A.J., Smith, C.K., Ballinger, M.N., Xia, M., et al., 2016. Inhibition of neutrophil extracellular trap formation after stem cell transplant by prostaglandin E2. Am. J. Respir. Crit. Care Med. 193, 186–197.

Farquharson, D., Butcher, J.P., Culshaw, S., 2012. Periodontitis, Porphyromonas, and the pathogenesis of rheumatoid arthritis. Mucosal Immunol. 5, 112–120.

Fuchs, T.A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., et al., 2007. Novel cell death program leads to neutrophil extracellular traps. J. Cell Biol. 176, 231–241.

Fuchs, T.A., Brill, A., Duerschmied, D., Schatzberg, D., Monestier, M., Myers, D.D., et al., 2010. Extracellular DNA traps promote thrombosis. Proc. Natl. Acad. Sci. U. S. A. 107, 15880–15885.

Grabcanovic-Musija, F., Obermayer, A., Stoiber, W., Krautgartner, W.-D., Steinbacher, P., et al., 2015. Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with airflow limitation. Respir. Res. 16, 59.

Guimarães-Costa, A.B., Nascimento, M.T.C., Wardini, A.B., Pinto-da-Silva, L.H., Saraiva, E.M., 2012. ETosis: a microbicidal mechanism beyond cell death. J. Parasitol. Res. 2012, 929743.

Gupta, A.K., Giaglis, S., Hasler, P., Hahn, S., 2014. Efficient neutrophil extracellular trap induction requires mobilization of both intracellular and extracellular calcium pools and is modulated by cyclosporine A. PLoS One 9, e97088.

Haase, H., Hebel, S., Engelhardt, G., Rink, L., 2016. Ethylmercury and Hg2+ induce the formation of neutrophil extracellular traps (NETs) by human neutrophil granulocytes. Arch. Toxicol. 90, 543–550.

Hakkim, A., Fürnrohr, B.G., Amann, K., Laube, B., Abed, U.A., Brinkmann, V., et al., 2010. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proc. Natl. Acad. Sci. U. S. A. 107, 9813–9818.

Halverson, T.W.R., Wilton, M., Poon, K.K.H., Petri, B., Lewenza, S., 2015. DNA is an antimicrobial component of neutrophil extracellular traps. PLoS Pathog. 11, e1004593.

Harsunen, M.H., Puff, R., D'Orlando, O., Giannopoulou, E., Lachmann, L., Beyerlein, A., et al., 2013. Reduced blood leukocyte and neutrophil numbers in the pathogenesis of type 1 diabetes. Horm. Metab. Res. 45, 467–470.

Haute, G.V., Caberlon, E., Squizani, E., de Mesquita, F.C., Pedrazza, L., Martha, B.A., et al., 2015. Gallic acid reduces the effect of LPS on apoptosis and inhibits the formation of neutrophil extracellular traps. Toxicol. In Vitro 30, 309–317.

Healy, L.D., Puy, C., Fernández, J.A., Mitrugno, A., Keshari, R.S., Taku, N.A., et al., 2017. Activated protein C inhibits neutrophil extracellular trap formation *in vitro* and activation *in vivo*. J. Biol. Chem. 292, 8616–8629.

Hemmers, S., Teijaro, J.R., Arandjelovic, S., Mowen, K.A., 2011. PAD4-mediated neutrophil extracellular trap formation is not required for immunity against influenza infection. PLoS One 6, e22043.

Hirsch, J.G., 1958. Bactericidal action of histone. J. Exp. Med. 108, 925–944.

Huang, Y.-M., Wang, H., Wang, C., Chen, M., Zhao, M.-H., 2015. Promotion of hypercoagulability in antineutrophil cytoplasmic antibody-associated vasculitis by C5a-induced tissue factor-expressing microparticles and neutrophil extracellular traps. Arthritis Rheumatol. 67, 2780–2790 (Hoboken N. J.).

Iba, T., Hashiguchi, N., Nagaoka, I., Tabe, Y., Murai, M., 2013. Neutrophil cell death in response to infection and its relation to coagulation. J. Intensive Care 1, 13.

Jaillon, S., Peri, G., Delneste, Y., Frémaux, I., Doni, A., Moalli, F., et al., 2007. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. J. Exp. Med. 204, 793–804.

Juneau, R.A., Pang, B., Weimer, K.E.D., Armbruster, C.E., Swords, W.E., 2011. Nontypeable Haemophilus influenzae initiates formation of neutrophil extracellular traps. Infect. Immun. 79, 431–438.

Kahlenberg, J.M., Carmona-Rivera, C., Smith, C.K., Kaplan, M.J., 2013. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. J. Immunol. 190, 1217–1226.

Kaplan, M.J., Radic, M., 2012. Neutrophil extracellular traps: double-edged swords of innate immunity. J. Immunol. 189, 2689–2695.

Kassebaum, N.J., Bernabé, E., Dahiya, M., Bhandari, B., Murray, C.J.L., Marcenes, W., 2014. Global burden of severe periodontitis in 1990–2010: a systematic review and meta-regression. J. Dent. Res. 93, 1045–1053.

Keshari, R.S., Jyoti, A., Dubey, M., Kothari, N., Kohli, M., Bogra, J., et al., 2012. Cytokines induced neutrophil extracellular traps formation: implication for the inflammatory disease condition. PLoS One 7, e48111.

Keshari, R.S., Verma, A., Barthwal, M.K., Dikshit, M., 2013. Reactive oxygen species-induced activation of ERK and p38 MAPK mediates PMA-induced NETs release from human neutrophils. J. Cell. Biochem. 114, 532–540.

Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J.S., et al., 2013. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci. Transl. Med. 5, 178ra40.

Kim, I., Hur, N.W., Shin, H.D., Park, B.L., Cheong, H.S., Bae, S.-C., 2008. Associations of DNase IV polymorphisms with autoantibodies in patients with systemic lupus erythematosus. Rheumatology 47, 996–999.

Knight, J.S., Subramanian, V., O'Dell, A.A., Yalavarthi, S., Zhao, W., Smith, C.K., et al., 2015. Peptidylarginine deiminase inhibition disrupts NET formation and

protects against kidney: skin and vascular disease in lupus-prone MRL/lpr mice. Ann. Rheum. Dis. 74, 2199–2206.
Korkmaz, B., Moreau, T., Gauthier, F., 2008. Neutrophil elastase, proteinase 3 and cathepsin G: Physicochemical properties, activity and physiopathological functions. Biochimie 90, 227–242.
Kusunoki, Y., Nakazawa, D., Shida, H., Hattanda, F., Miyoshi, A., Masuda, S., et al., 2016. Peptidylarginine deiminase inhibitor suppresses neutrophil extracellular trap formation and MPO-ANCA production. Front. Immunol. 7, 227.
Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y.-H., Homey, B., et al., 2007. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 449, 564–569.
Lapponi, M.J., Carestia, A., Landoni, V.I., Rivadeneyra, L., Etulain, J., Negrotto, S., et al., 2013. Regulation of neutrophil extracellular trap formation by anti-inflammatory drugs. J. Pharmacol. Exp. Ther. 345, 430–437.
Li, P., Li, M., Lindberg, M.R., Kennett, M.J., Xiong, N., Wang, Y., 2010. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J. Exp. Med. 207, 1853–1862.
Liu, Z., Davidson, A., 2012. Taming lupus—a new understanding of pathogenesis is leading to clinical advances. Nat. Med. 18, 871–882.
Luo, H.R., Loison, F., 2008. Constitutive neutrophil apoptosis: mechanisms and regulation. Am. J. Hematol. 83, 288–295.
Ma, C., Jiao, Y., Zhang, J., Yang, Q., Zhang, Z., Shen, Y., et al., 2012. Elevated plasma level of HMGB1 is associated with disease activity and combined alterations with IFN-α and TNF-α in systemic lupus erythematosus. Rheumatol. Int. 32, 395–402.
Majewski, P., Majchrzak-Gorecka, M., Grygier, B., Skrzeczynska-Moncznik, J., Osiecka, O., Cichy, J., 2016. Inhibitors of serine proteases in regulating the production and function of neutrophil extracellular traps. Front. Immunol. 7, 261.
Malachowa, N., Kobayashi, S.D., Freedman, B., Dorward, D.W., DeLeo, F.R., 2013. Staphylococcus aureus leukotoxin GH promotes formation of neutrophil extracellular traps. J. Immunol. 191, 6022–6029.
Marcos, V., Zhou, Z., Yildirim, A.O., Bohla, A., Hector, A., Vitkov, L., et al., 2010. CXCR2 mediates NADPH oxidase-independent neutrophil extracellular trap formation in cystic fibrosis airway inflammation. Nat. Med. 16, 1018–1023.
Marin-Esteban, V., Turbica, I., Dufour, G., Semiramoth, N., Gleizes, A., Gorges, R., et al., 2012. Afa/Dr diffusely adhering *Escherichia coli* strain C1845 induces neutrophil extracellular traps that kill bacteria and damage human enterocyte-like cells. Infect. Immun. 80, 1891–1899.
Massberg, S., Grahl, L., von Bruehl, M.-L., Manukyan, D., Pfeiler, S., Goosmann, C., et al., 2010. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat. Med. 16, 887–896.
Matthews, J.B., Wright, H.J., Roberts, A., Cooper, P.R., Chapple, I.L.C., 2007. Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. Clin. Exp. Immunol. 147, 255–264.
McIlroy, D.J., Jarnicki, A.G., Au, G.G., Lott, N., Smith, D.W., Hansbro, P.M., Balogh, Z.J., 2014. Mitochondrial DNA neutrophil extracellular traps are formed after trauma and subsequent surgery. J. Crit. Care 29 (1133), e1–5.
Metzler, K.D., Fuchs, T.A., Nauseef, W.M., Reumaux, D., Roesler, J., Schulze, I., et al., 2011. Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity. Blood 117, 953–959.
Miyoshi, A., Yamada, M., Shida, H., Nakazawa, D., Kusunoki, Y., Nakamura, A., et al., 2016. Circulating neutrophil extracellular trap levels in well-controlled Type 2 diabetes and pathway involved in their formation induced by high-dose glucose. Pathobiology 83, 243–251.
Mohammed, B.M., Fisher, B.J., Kraskauskas, D., Farkas, D., Brophy, D.F., Fowler, A.A., Natarajan, R., 2013. Vitamin C: a novel regulator of neutrophil extracellular trap formation. Nutrients 5, 3131–3151.
Narasaraju, T., Yang, E., Samy, R.P., Ng, H.H., Poh, W.P., Liew, A.-A., et al., 2011. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. Am. J. Pathol. 179, 199–210.
Neeli, I., Khan, S.N., Radic, M., 2008. Histone deimination as a response to inflammatory stimuli in neutrophils. J. Immunol. 180, 1895–1902.
Neumann, A., Brogden, G., Jerjomiceva, N., Brodesser, S., Naim, H.Y., von Köckritz-Blickwede, M., 2014a. Lipid alterations in human blood-derived neutrophils lead to formation of neutrophil extracellular traps. Eur. J. Cell Biol. 93, 347–354.
Neumann, A., Völlger, L., Berends, E.T.M., Molhoek, E.M., Stapels, D.A.C., Midon, M., et al., 2014b. Novel role of the antimicrobial peptide LL-37 in the protection of neutrophil extracellular traps against degradation by bacterial nucleases. J. Innate Immun. 6, 860–868.
Nickerson, K.M., Cullen, J.L., Kashgarian, M., Shlomchik, M.J., 2013. Exacerbated autoimmunity in the absence of TLR9 in MRL.Fas(lpr) mice depends on Ifnar1. J. Immunol. 190, 3889–3894.
Padgett, L.E., Broniowska, K.A., Hansen, P.A., Corbett, J.A., Tse, H.M., 2013. The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. Ann. N. Y. Acad. Sci. 1281, 16–35.
Palić, D., Ostojić, J., Andreasen, C.B., Roth, J.A., 2007. Fish cast NETs: neutrophil extracellular traps are released from fish neutrophils. Dev. Comp. Immunol. 31, 805–816.
Palmer, L.J., Cooper, P.R., Ling, M.R., Wright, H.J., Huissoon, A., Chapple, I.L.C., 2012a. Hypochlorous acid regulates neutrophil extracellular trap release in humans. Clin. Exp. Immunol. 167, 261–268.
Palmer, L.J., Chapple, I.L.C., Wright, H.J., Roberts, A., Cooper, P.R., 2012b. Extracellular deoxyribonuclease production by periodontal bacteria. J. Periodontal Res. 47, 439–445.
Papayannopoulos, V., Metzler, K.D., Hakkim, A., Zychlinsky, A., 2010. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J. Cell Biol. 191, 677–691.
Papayannopoulos, V., Staab, D., Zychlinsky, A., 2011. Neutrophil elastase enhances sputum solubilization in cystic fibrosis patients receiving DNase therapy. PLoS One 6, e28526.
Parker, H., Albrett, A.M., Kettle, A.J., Winterbourn, C.C., 2012. Myeloperoxidase associated with neutrophil extracellular traps is active and mediates bacterial killing in the presence of hydrogen peroxide. J. Leukoc. Biol. 91, 369–376.
Pilsczek, F.H., Salina, D., Poon, K.K.H., Fahey, C., Yipp, B.G., Sibley, C.D., et al., 2010. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J. Immunol. 185, 7413–7425.
Ramos-Kichik, V., Mondragón-Flores, R., Mondragón-Castelán, M., Gonzalez-Pozos, S., Muñiz-Hernandez, S., Rojas-Espinosa, O., et al., 2009. Neutrophil extracellular traps are induced by *Mycobacterium tuberculosis*. Tuberculosis 89, 29–37 (Edinb).
Rohrbach, A.S., Hemmers, S., Arandjelovic, S., Corr, M., Mowen, K.A., 2012. PAD4 is not essential for disease in the K/BxN murine autoantibody-mediated model of arthritis. Arthritis Res. Ther. 14, R104.
Rossaint, J., Herter, J.M., Van Aken, H., Napirei, M., Döring, Y., Weber, C., et al., 2014. Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap-mediated sterile inflammation. Blood 123, 2573–2584.
Saffarzadeh, M., Juenemann, C., Queisser, M.A., Lochnit, G., Barreto, G., Galuska, S.P., et al., 2012. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PLoS One 7, e32366.
Schürmann, N., Forrer, P., Casse, O., Li, J., Felmy, B., Burgener, A.-V., et al., 2017. Myeloperoxidase targets oxidative host attacks to Salmonella and prevents collateral tissue damage. Nat. Microbiol. 2, 16268.
Schorn, C., Janko, C., Latzko, M., Chaurio, R., Schett, G., Herrmann, M., 2012. Monosodium urate crystals induce extracellular DNA traps in neutrophils, eosinophils, and basophils but not in mononuclear cells. Front. Immunol. 3, 277.
Sha, L.-L., Wang, H., Wang, C., Peng, H.-Y., Chen, M., Zhao, M.-H., 2016. Autophagy is induced by anti-neutrophil cytoplasmic Abs and promotes neutrophil extracellular traps formation. Innate Immun. 22, 658–665.
Shimomura, Y., Suga, M., Kuriyama, N., Nakamura, T., Sakai, T., Kato, Y., et al., 2016. Recombinant human thrombomodulin inhibits neutrophil extracellular trap formation *in vitro*. J. Intensive Care 4, 48.
Shin, H.D., Park, B.L., Kim, L.H., Lee, H.-S., Kim, T.-Y., Bae, S.-C., 2004. Common DNase I polymorphism associated with autoantibody production among systemic lupus erythematosus patients. Hum. Mol. Genet. 13, 2343–2350.
Shishikura, K., Horiuchi, T., Sakata, N., Trinh, D.-A., Shirakawa, R., Kimura, T., et al., 2016. Prostaglandin E2 inhibits neutrophil extracellular trap formation through production of cyclic AMP. Br. J. Pharmacol. 173, 319–331.
Skopelja, S., Hamilton, B.J., Jones, J.D., Yang, M.-L., Mamula, M., Ashare, A., et al., 2016. The role for neutrophil extracellular traps in cystic fibrosis autoimmunity. JCI Insight 1, e88912.
Stoehr, A.D., Schoen, C.T., Mertes, M.M.M., Eiglmeier, S., Holecska, V., Lorenz, A.K., et al., 2011. TLR9 in peritoneal B-1b cells is essential for production of protective self-reactive IgM to control Th17 cells and severe autoimmunity. J. Immunol. 187, 2953–2965.
Sur Chowdhury, C., Giaglis, S., Walker, U.A., Buser, A., Hahn, S., Hasler, P., 2014. Enhanced neutrophil extracellular trap generation in rheumatoid arthritis: analysis of underlying signal transduction pathways and potential diagnostic utility. Arthritis Res. Ther. 16, R122.
Szekanecz, Z., Soós, L., Szabó, Z., Fekete, A., Kapitány, A., Végvári, A., et al., 2008. Anti-citrullinated protein antibodies in rheumatoid arthritis: as good as it gets? Clin. Rev. Allergy Immunol. 34, 26–31.
Tadie, J.-M., Bae, H.-B., Jiang, S., Park, D.W., Bell, C.P., Yang, H., et al., 2013. HMGB1 promotes neutrophil extracellular trap formation through interactions with Toll-like receptor 4. Am. J. Physiol. Lung Cell. Mol. Physiol. 304, L342–349.
Tripathi, S., Verma, A., Kim, E.-J., White, M.R., Hartshorn, K.L., 2014. LL-37 modulates human neutrophil responses to influenza A virus. J. Leukoc. Biol. 96, 931–938.
Urban, C.F., Ermert, D., Schmid, M., Abu-Abed, U., Goosmann, C., Nacken, W., et al., 2009. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defence against *Candida albicans*. PLoS Pathog. 5, e1000639.
Valle, A., Giamporcaro, G.M., Scavini, M., Stabilini, A., Grogan, P., Bianconi, E., et al., 2013. Reduction of circulating neutrophils precedes and accompanies Type 1 diabetes. Diabetes 62, 2072–2077.
Ventura-Juarez, J., Campos-Esparza, M., Pacheco-Yepez, J., López-Blanco, J.A., Adabache-Ortíz, A., Silva-Briano, M., Campos-Rodríguez, R., 2016. Entamoeba histolytica induces human neutrophils to form NETs. Parasite Immunol. 38, 503–509.
von Brühl, M.-L., Stark, K., Steinhart, A., Chandraratne, S., Konrad, I., Lorenz, M., et al., 2012. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice *in vivo*. J. Exp. Med. 209, 819–835.
Wang, Y., Chen, Y., Xin, L., Beverley, S.M., Carlsen, E.D., Popov, V., et al., 2011. Differential microbicidal effects of human histone proteins H2A and H2B on Leishmania promastigotes and amastigotes. Infect. Immun. 79, 1124–1133.
Wang, Y., Xiao, Y., Zhong, L., Ye, D., Zhang, J., Tu, Y., et al., 2014. Increased neutrophil elastase and proteinase 3 and augmented NETosis are closely associated with β-cell autoimmunity in patients with type 1 diabetes. Diabetes 63, 4239–4248.
Wang, H., Li, T., Chen, S., Gu, Y., Ye, S., 2015. Neutrophil extracellular trap mitochondrial DNA and its autoantibody in systemic *Lupus Erythematosus* and a

proof-of-concept trial of metformin. Arthritis Rheumatol. 67, 3190–3200 (Hoboken N.J.).
Wartha, F., Beiter, K., Albiger, B., Fernebro, J., Zychlinsky, A., Normark, S., Henriques-Normark, B., 2007. Capsule and D-alanylated lipoteichoic acids protect Streptococcus pneumoniae against neutrophil extracellular traps. Cell. Microbiol. 9, 1162–1171.
Weinrauch, Y., Drujan, D., Shapiro, S.D., Weiss, J., Zychlinsky, A., 2002. Neutrophil elastase targets virulence factors of enterobacteria. Nature 417, 91–94.
White, P., Cooper, P., Milward, M., Chapple, I., 2014. Differential activation of neutrophil extracellular traps by specific periodontal bacteria. Free Radic. Biol. Med. 75 (Suppl. 1), S53.
Wright, H.J., Matthews, J.B., Chapple, I.L.C., Ling-Mountford, N., Cooper, P.R., 2008. Periodontitis associates with a type 1 IFN signature in peripheral blood neutrophils. J. Immunol. 181, 5775–5784.
Xu, J., Zhang, X., Pelayo, R., Monestier, M., Ammollo, C.T., Semeraro, F., et al., 2009. Extracellular histones are major mediators of death in sepsis. Nat. Med. 15, 1318–1321.
Yang, H., Biermann, M.H., Brauner, J.M., Liu, Y., Zhao, Y., Herrmann, M., 2016. New insights into neutrophil extracellular traps: mechanisms of formation and role in inflammation. Front. Immunol. 7, 302.
Yasutomo, K., Horiuchi, T., Kagami, S., Tsukamoto, H., Hashimura, C., Urushihara, M., Kuroda, Y., 2001. Mutation of DNASE1 in people with systemic lupus erythematosus. Nat. Genet. 28, 313–314.
Yipp, B.G., Kubes, P., 2013. NETosis: how vital is it? Blood 122, 2784–2794.
Yoo, D., Winn, M., Pang, L., Moskowitz, S.M., Malech, H.L., Leto, T.L., Rada, B., 2014. Release of cystic fibrosis airway inflammatory markers from *Pseudomonas aeruginosa*-stimulated human neutrophils involves NADPH oxidase-dependent extracellular DNA trap formation. J. Immunol. 192, 4728–4738.
Yousefi, S., Mihalache, C., Kozlowski, E., Schmid, I., Simon, H.U., 2009. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. Cell Death Differ. 16, 1438–1444.
Zabieglo, K., Majewski, P., Majchrzak-Gorecka, M., Wlodarczyk, A., Grygier, B., Zegar, A., et al., 2015. The inhibitory effect of secretory leukocyte protease inhibitor (SLPI) on formation of neutrophil extracellular traps. J. Leukoc. Biol. 98, 99–106.