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NEW INSIGHTS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETs) IN AUTOIMMUNITY AND ITS APPLICATIONS

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ABSTRACT. Neutrophils are thought to be the initial line of defence for the immune system. They can be found in tissue for up to seven days in the bloodstream, where they can survive for six to eight hours. Neutrophils defend their hosts by phagocytosis, degranulation, the creation of cytokines, and, most recently studied, the development of NETs.NETosis is a sort of neutrophil specific cell death marked by the release of the enormous web-like structures referred to as NETs. Two pathways of NETosis are known to date i) Suicidal/Classical Pathway which results in cell death, ii) Vital NETosis Pathway, in which the cell retains not only it's viability but also many of its effector activities are retained. Over the past decade and a half of research, it has been shown that NETs positively aid the body's immune system in defending it from the pathogens. However, it has also been shown that neutrophils and NETs are not always beneficial to one's body. They have been found at the sites of a multitude of diseases where they contribute to the pathogenesis of the disease by various means like presenting self-antigen to autoantibodies. In this review, the basic mechanism of NETosis, as well its role in some autoimmune diseases including Rheumatoid arthritis, Systemic Lupus Erythematosus, Type-1 Diabetes etc., along with various clinical applications has been discussed.

Keywords: *Immune system; Neutrophils; NETs; autoimmune diseases*

OTOİMMÜNİTEDE NÖTROFİL HÜCRE DIŞI TUZAKLAR (NETs) VE UYGULAMALARINA İLİŞKİN YENİ ANLAYIŞLAR

ÖZET. Nötrofillerin bağışıklık sisteminin ilk savunma hattı olduğu düşünülmektedir. Kan dolaşımında dokuda yedi güne kadar bulunabilirler ve burada altı ila sekiz saat boyunca hayatta kalabilirler. Nötrofiller konakçılarını fagositoz, degranülasyon, sitokinlerin oluşturulması ve en son incelenen NET'lerin gelişimi yoluyla savunurlar. NETosis, NET'ler olarak adlandırılan devasa ağ benzeri yapıların salınmasıyla işaretlenen bir tür nötrofil spesifik hücre ölümüdür. Bugüne kadar NETosis'in iki yolu bilinmektedir: i) Hücre ölümüyle sonuçlanan İntihar/Klasik Yol, ii) Hücrenin sadece canlılığını korumakla kalmayıp aynı zamanda efektör aktivitelerinin çoğunun da muhafaza edildiği Vital NETosis Yolu. Geçtiğimiz on beş yılda yapılan araştırmalarda, NET'lerin vücudun bağışıklık sistemine patojenlere karşı savunmada olumlu bir şekilde yardımcı olduğu gösterilmiştir. Ancak nötrofillerin ve NET'lerin kişinin vücuduna her zaman faydalı olmadığı da gösterilmiştir. Kendi antijenini otoantikorlara sunmak gibi çeşitli yollarla hastalığın patogenezine olan katkıları, hastalıkların birçok alanında bulunmuştur. Bu derlemede NETosis'in temel mekanizması, Romatoid artrit, Sistemik Lupus Eritematozus, Tip-1 Diyabet gibi bazı otoimmün hastalıklardaki rolü ve çeşitli klinik uygulamaları tartışılmıştır.

Anahtar Kelimeler: *Bağışıklık sistemi; Nötrofiller; NET'ler; Otoimmün Hastalıklar*

INTRODUCTION

Immune Response

Our body has an in-built defence mechanism to combat nonself or foreign substances. Antigens (typically proteins) on the surface of foreign substances or microorganisms, such as bacteria or viruses, are recognised by the immune system, which fights and destroys, or tries to destroy them. Antigen triggers the immune response, which is the body's reaction to foreign substances [1]. This reaction defends the body against disease-causing viruses, fungi, bacteria, and parasites. When transplanted organs are recognised as foreign, they can trigger an immunological reaction [2]. The immunological reaction is divided into two arms: the humoral arm of immunity and cellular arm of the immunity. Humoral immunity is mediated by B cell receptors (BCR). The B cell receptors are released from the B cells in response to an antigen and are called Antibody. Cellular immunity mediated by T cell receptor (TCR). T cells are of two types T helper cells (T_H) and T cytotoxic cells (T_C). The B cells and T cells collaborate to bring about the immune response to fight the antigen [3].

The immune system is a collection of biological structures and activities that defends an organism from disease. An immune system must be able to recognise a wide range of agents, from viruses to parasitic worms, and separate them from the organism's own healthy tissue in order to function correctly. Pathogens can change and adapt quickly in order to avoid being detected and neutralised by the immune system. As a result, several defence mechanisms to recognise and kill viruses have evolved. Even small unicellular organisms like bacteria have a primitive immune system in the form of enzymes that protect them from bacteriophage infections [4]. Phagocytosis, antimicrobial peptides known as defensins, and the complement system, which evolved in ancient eukaryotes and is still present in modern descendants such as plants and insects, are examples of basic immunological systems [5].

In this review article, it has been discussed about Neutrophil suicide which is commonly known as NETosis. Process NETosis was actually observed after the discovery of phorbol 12-myristate 13-acetate (PMA) and it also stimulates neutrophils [7]. A critical step in NETosis is the release of certain proteins from granules into the cytosol. Azurophil granules contain the protein complex "azurosome" and ROS have been shown to cause azurosome dissociation, which causes serine proteases and MPO to be released from the granules and enter the cytosol [15]. Cytoskeletal elements are then broken down by serine proteases, most notably NE, allowing NETosis to take place [16]. There are majorly 2 pathways of NETosis discussed over here: (i) Suicidal NETosis (ii) Vital NETosis.

Here, it has also been discussed about how neutrophil leads to the condition of pathogenesis. Numerous systemic autoimmune diseases and autoinflammatory syndromes, such as adult-onset Still's disease (AOSD), which is brought on by innate immunity, and rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ANCA-associated vasculitis (AAV), which is brought on by adaptive immunity, have been linked to the pathogenesis by neutrophils. The involvement of neutrophils in inflammatory autoimmune illness is still unknown [40].

Neutrophil extracellular traps are hypothesised to participate in an autoimmune response as they get exposed to the intracellular endogenous components to the immune system. This article mainly discusses how NET is involved in activation of autoimmune response

Neutrophils

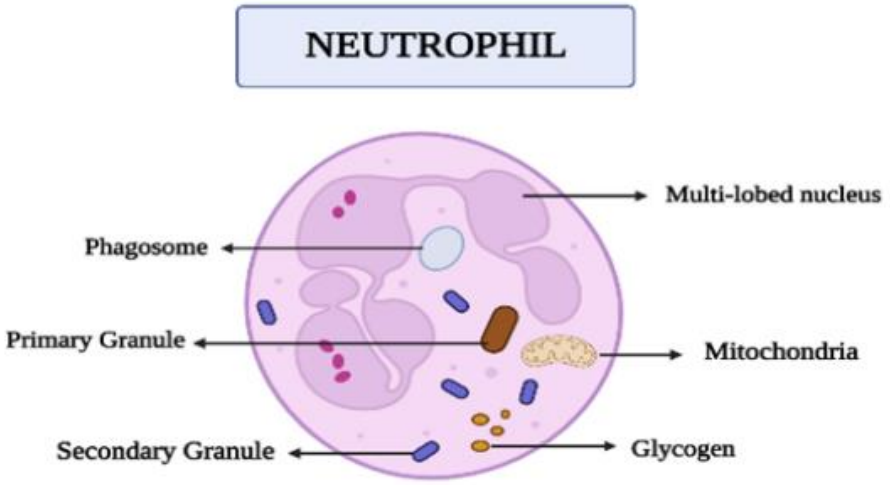

Fig.1. *Showing neutrophil and its components*

Neutrophils are majorly found in white blood cells in circulation and are essential in maintaining our health. Neutrophils differentiate from hematopoietic stem cells and leave the bone marrow when they are terminally developed [6]. They spend their brief existence circulating in the bloodstream. Components of neutrophil mainly include phagosome, primary granule, secondary granule, multi-lobed nucleus, mitochondria and glycogen as illustrated in Fig.1. In particular, only neutrophils possess multi-lobed nucleus.

Neutrophils cover 60-65% of white blood cells in peripheral blood and are involved in many physiological and pathological processes of our body [4]. Neutrophil has a significant position in the first line of defence of the immune system against bacterial and viral infections by the means of phagocytosis and activation of intracellular proteins [5]. Additionally, under pathological conditions, neutrophils are also capable of releasing traps called NETs.

Neutrophil Extracellular Trap [NET]

NETs consist of extracellular DNA fibres which further includes histone proteins and cytoplasmic granules [6]. NETs were first found in 1996 and further described by Brinkmann in 2004 as a form of the innate response to pathogen invasion that can trap pathogens, destroy bacterial virulence factors, and kill bacteria, who also later coined the term "NETosis" to describe

the process [7, 8, 9]. Neutrophils are short-lived granulocytes that serve as the body's first line of defence against infections. Phagocytosis, degranulation, the formation of reactive oxygen species (ROS), and the creation of cytokines and chemokines to provide additional immune cells in order to maximise the host's immunological response are all employed for this process [6,10].

The innate immune system's major cells are neutrophils. The creation of NETs is one of the neutrophils' methods of action. The discovery of the network has spawned a new branch of granulocyte research. Nuclear chromatin and granular antimicrobial proteins linked to nuclear histones make up NETs. They make excellent germ-holding scaffolds. NETs are used to catch and kill disease-causing microbes such as bacteria, fungus, viruses, and protozoa (Fig.2). The confinement of viruses inside DNA strands inhibits their spread and boosts antimicrobial agent concentrations at the infection's focal point [6]. The creation of a NET, also known as NETosis, is a specific type of cell death. NET is a web-like trap that engulfs the cell inside it through NETosis further leads to cell death.

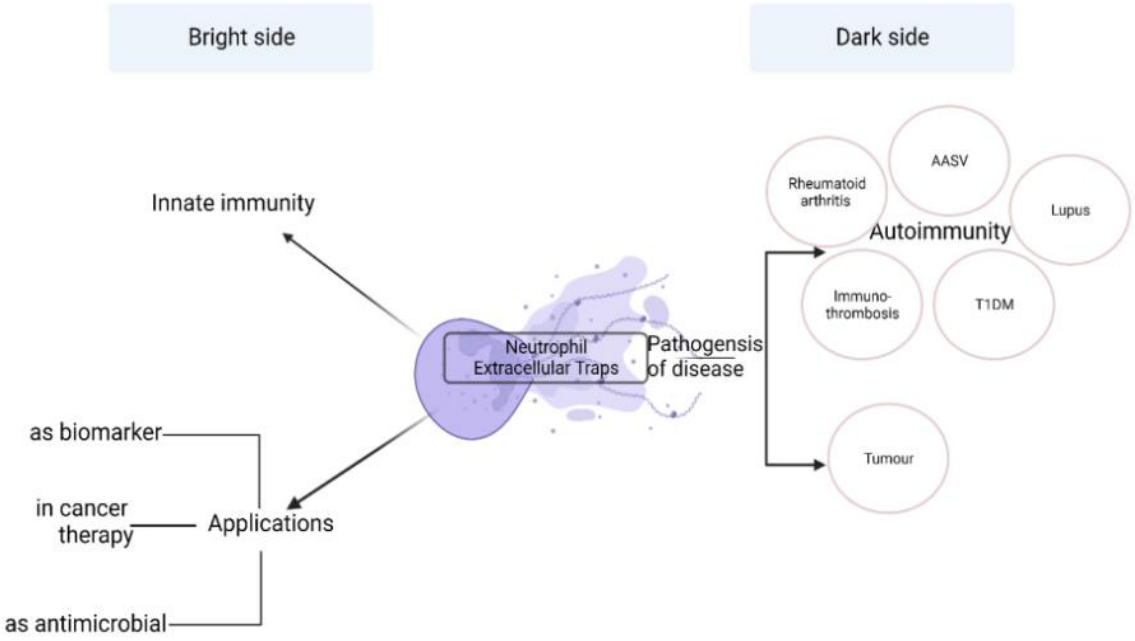

Fig.2. *Schematic diagram of various sides of NETs*

NETs are extracellular fibre networks that bind pathogens and are mostly made up of neutrophil DNA [8]. Neutrophils are the immune system's first line of protection against infection, and they are thought to kill pathogens via two mechanisms: microbe engulfment and antimicrobial release. A new third function was identified in 2004: the development of NETs. Neutrophils use NETs to destroy extracellular infections while causing the least amount of damage to the host cells [11].

Neutrophils, in addition to physical barriers, are considered part of the immune system's first line of protection. They can be found in the bloodstream, where they have a lifespan of 6–8 hours, and in tissue, where they have a 7-day existence [12]. Phagocytosis, degranulation, cytokine production, and, most recently, NET generation are all strategies neutrophils use to defend their host[8]. NETs are DNA structures that are formed when chromatin decondense and spreads, occupying three to five times the volume of compact chromatin. Histones and over 30 components of primary and secondary granules, including bactericidal components like elastase, myeloperoxidase, cathepsin G, lactoferrin, pentraxin 3, gelatinase, proteinase 3, LL37, and peptidoglycan-binding proteins, cling to NETs and with this bactericidal activity they are able to destroy virulence factors [6,13].

NET - Structure and Composition

NETs are made up of DNA stretches and globular protein domains with sizes of 15-17 nm and 25 nm, respectively, according to high-resolution scanning electron microscopy. They clump together to form bigger threads with a diameter of 50 nanometer [8]. Under current parameters, NETs can also form much larger structures, reaching hundreds of nanometers in length and width [14]. NETs consist of proteins from azurophilic granules (cathepsin G, neutrophil elastase and myeloperoxidase), specialised granules (lactoferrin), tertiary granules (gelatinase), and the cytoplasm, according to immunofluorescence analysis.Programmed Cell Death of Neutrophil is NETosis is characterised by the secretion of NETs which are large web-like structures. NETs consist of chromatin fibres that make up the DNA with diameters of 15-17 nm. Histones H1, H2A, H2B, H3 and H4 are also composed of proteins from granules (i.e., Neutrophil elastase (NE) cathepsin G, and Myeloperoxidase (MPO), and some specific granules like lactoferrin, gelatinase which is the tertiary granules.NE (Neutrophil elastase) is a serine protease that eliminates bacteria and MPO that activates the oxidation of halides by hydrogen peroxide [8].

Mechanism of NETosis

Neutrophil suicide, which differs from necrosis and apoptosis, was initially observed in 1996 after phorbol 12-myristate 13-acetate (PMA) was used to stimulate neutrophils [7]. The release of decondensed chromatin into the cytoplasm, as well as the breakdown of nuclear and granular membranes, were characteristics of this type of cell death. In 2004, Zychlinsky and colleagues discovered that neutrophil suicide resulted in the production of enormous web-like structures made up of decondensed chromatin and neutrophil antimicrobial components, which they termed neutrophil extracellular traps [8].

The release of certain proteins from granules into the cytosol is a crucial event in NETosis. Azurophil granules contain the protein complex "azurosome," which contains eight different proteins, three of which are highly homologous serine proteases – neutrophil elastase (NE), cathepsin G, and azurocidin – as well as myeloperoxidase (MPO), an enzyme that uses chlorine and hydrogen peroxide as substrates to produce hypochlorite anion. ROS have been demonstrated to cause azurosome dissociation, resulting in the release of serine proteases and MPO from the granules into the cytosol [15]. Serine proteases (most notably NE) degrade cytoskeletal components, allowing NETosis to occur [16].

They then move to the nucleus, where they break down lamin and histones, causing chromatin decondensation and the nuclear envelope to be destroyed. MPO is involved in the dissociation of the azurosome as well as the release of proteases from the granule [15]. MPO's enzymatic activity isn't necessary at this stage, which is surprising. This heme-containing protein most likely functions as an intracellular hydrogen peroxide receptor. MPO activity, on the other hand, is essential for NETosis because the hypochlorite anion increases neutrophil elastase activity [15]. It's worth noting that the ROS-dependent release of proteins from granules during NETosis is strikingly similar to the ROS-dependent permeabilization of lysosomes and release of cathepsins from them are seen in various types of necrosis [17]. This whole procedure of NETosis mechanism is illustrated in Fig. 3.

Pathways

Classical or suicidal NETosis, which results in cell death, and vital NETo**sis,** in which the cell retains not only viability but also many of its effector activities, are the two types of NETosis currently described.

Suicidal NETosis

Reactive Oxygen Species (ROS)

The general belief, two major observations suggest that ROS play a key role in the classic suicidal NETosis pathway: (1) Neutrophils from patients with the chronic granulomatous disease (CGD), which are unable to complete the oxidative burst, have significantly diminished NET formation capacity. This is true regardless of the mutation that causes a faulty PHOX complex. Infections are common in CGD patients, and they are often severe and chronic [18,19]. Furthermore, H_2O_2 therapy stopped the creation of NETs in CGD patients' neutrophils, which was caused by the PHOX complex [18]. (2) ROS scavengers like N-acetylcysteine and Trolox are said to prevent NETosis [18,20]. In addition, its unknown how ROS plays a role in the dissolution of the nuclear envelope or the mixing of NET components. According to certain research, ROS have a direct role in the morphologic alterations that occur during NETosis [21]. ROS can also inactivate caspases, which inhibit apoptosis and promote autophagy. This causes cellular membranes to dissolve [22]. These two options are not mutually exclusive: each of them can act independently under certain experimental conditions. There is currently mounting evidence that some stimuli can cause NETosis without the involvement of NADPH oxidase. Winterbourn and colleagues investigated the oxidant-independent release of NETs in-depth [23].

Peptidyl Arginine Deiminase 4 (PAD4)

Peptidyl arginine deiminase 4 converts arginine residues to citrulline in polypeptides, removing the protein's positive charge. As a result, histone citrullination reduces nucleosome stability [24, 25]. The loss of positive charges leads the compact structure of chromatin to open up, allowing chromatin to decondense and disperse in the form of NETs. In vivo, neutrophils from mice lacking PAD4 have a reduced ability to form NETs and are more prone to severe skin infections [26, 27]. PAD4 deficiency, on the other hand, has no effect on influenza virus-related lung infections.

Vital NETosis

There are methods of DNA release, in addition to the suicidal NETosis mentioned above, where neutrophils retain their viability and normal effector roles [28]. These processes were named "vital NETosis" by researchers. However, the Cell Death Nomenclature Committee recommended against using the term NETosis for processes that are not related to cell death in 2018 [29]. The crucial release of chromatin was initially seen in a system containing neutrophils and platelets triggered by LPS via TLR4 [14].

The rate of NETosis was substantially faster in this situation than when induced by PMA, and the role of NADPH oxidase and ROS was not investigated. Unfortunately, this intriguing idea, which has clear physiological implications, has not been further examined. In this scenario, NETosis was induced by opsonizing bacteria, interacting with TLR2, and activating the complement system. Surprisingly, nuclear-free neutrophils were capable of chemotaxis and phagocytosis of bacteria after NETosis [14].

In eosinophils and neutrophils primed with pro-inflammatory cytokines IL-5/IFN-α or GM-CSF and activated with LPS, massive and extremely fast release of mitochondrial DNA (mtDNA) was seen without loss of viability [30,31]. The activity of NADPH oxidase was required for this action in both types of granulocytes. In the case of basophils, a similar occurrence was found [32]. In response to oligodeoxynucleotides, essential mtDNA release was identified in B and T lymphocytes, as well as in natural killer cells (NK cells), [33].

Unlike granulocytes, lymphocytes did not require NADPH oxidase to release mtDNA. The functional relevance of extracellular mtDNA is still a mystery. The low mitochondrial content of neutrophils, particularly eosinophils, makes the creation of functional NETs highly unlikely. Extracellular mtDNA in lymphocytes did not contain lytic enzymes and most likely served a signalling function. mtDNA enhanced type I interferon production and secretion by peripheral blood mononuclear cells in particular [33].

Extracellular mtDNA (typically oxidised) is discovered in the blood in a variety of diseases, including systemic lupus erythematosus, an inflammatory illness in which NETosis plays a major part in the pathogenesis [34].

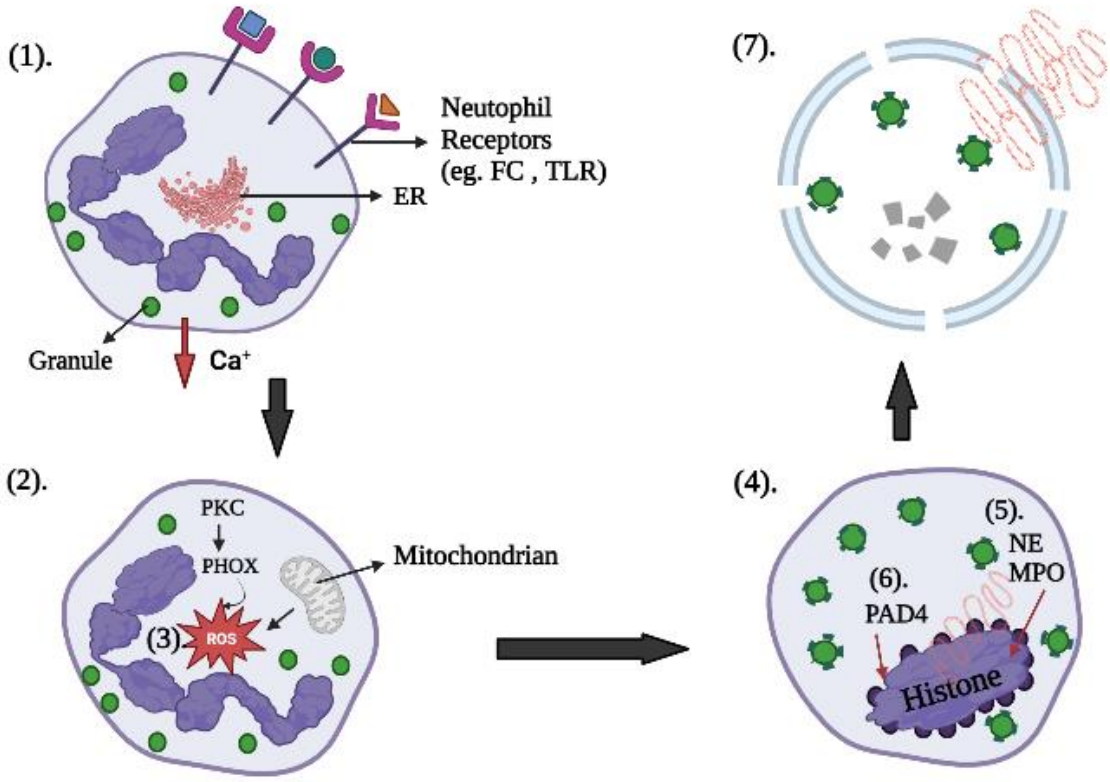

Fig.3. *Showing Mechanism of NETosis (1). NETOSIS is initialized by several stimuli (E.G., FUNGI, VIRUSES, BACTERIA) binding to neutrophil receptors such as FC Receptors, Toll - like receptors), Which activate the endoplasmic reticulum to release stored Calcium ions. (2). Increased cytoplasmic calcium levels stimulate PKC activity, causing NADPH oxidase to form a functional complex (PHOX). (3). PHOX (or, alternatively, the mitochondrial respiratory chain) then generates ROS (Reactive oxygen species) (4). ROS generation causes granule and nuclear envelope rupture. (5) In the meantime, NE and MPO are translocated to the nucleus. (6). (VI) As a result, histone deimination and chromatin decondensation help to form NETs (7). Finally, the rupture of the plasma membrane causes.*

Mitochondrial DNA

As previously noted, ROS are essential for several forms of NETosis [35]. In mammals, both the mitochondrial respiration chain and NADPH oxidase contribute to the production of reactive oxygen species (ROS) [36]. *In vivo* suppression of mitochondrial ROS generation has been shown to lower intracellular ROS levels and NETosis [34]. To promote neutrophils and mitochondrial ROS production, RNP immune complexes (RNP ICs) were utilised. Mitochondria became hyperpolarized, translocated to the cell surface, and were found within NETs that had been ejected. *In vitro*, mitochondrial ROS also oxidised mitochondrial DNA (mtDNA), which is proinflammatory. Oxidised mtDNA promoted inflammation and type I IFN production in mice via a route involving the DNA sensor STING [34, 37].

Abnormal NETosis and NET clearance abnormalities were reported to enhance the generation and release of type I IFN in patients with systemic lupus erythematosus (SLE) [38]. Patients with CGD, on the other hand, have a higher chance of developing SLE, despite the fact that they lack functioning NADPH oxidase activity [19], the principal generator of ROS in activated healthy neutrophils. On the basis of this finding, one might wonder if increased NETosis is a factor in the etiopathogenesis of SLE. Instead, a lack of NET clearance is expected to exacerbate antinuclear autoimmunity in SLE patients [38, 39].

Neutrophils and Autoimmunity

Autoimmunity is described as an immune response that results in a reaction with a self-antigen, which otherwise is any molecule that is a normal body element/cell and is incapable of triggering an immune response. In recent years, neutrophils have been implicated in the pathogenesis of a number of systemic autoimmune diseases and autoinflammatory syndromes, including adult-onset Still's disease (AOSD), which is caused by innate immunity, and rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ANCA-associated vasculitis (AAV), which is caused by adaptive immunity. The exact involvement of neutrophils in inflammatory autoimmune illness is mostly unknown [40].

Neutrophil extracellular traps are suspected to participate in an autoimmune response as they expose the intracellular endogenous components to the immune system. Most of the proteins in these structures, notably myeloperoxidase (MPO) and peptidylarginine-deiminase 4 (PADI4), have been demonstrated to greatly increase the autoantigenic burden, making them effective autoantigens for autoantibody formation [41].

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune illness that causes pain, inflammation, and damage to joints all over the body. Neutrophils in RA patients are prepared for the production of reactive oxygen species hence they behave differently than neutrophils in healthy people [42].

Because of their propensity to generate degradative enzymes and reactive oxygen species, neutrophils have the most cytotoxic potential of all the cells implicated in the pathophysiology of rheumatoid arthritis (RA) [43]. Neutrophils have serine proteases in their cytoplasmic granules, which can be released when they are stimulated. Anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) immune complexes in RA joints can trigger neutrophil degranulation via FcγR [44]. Activated neutrophils, in addition to secreting proteases, regulate the adaptive immune response in the same way that macrophages and dendritic cells do. TNF, B cell-activating factor (BAFF) [45], and receptor activator of nuclear factor kappa B ligand (RANKL) [46] are all inflammatory TNF family cytokines secreted by RA synovial fluid neutrophils [47, 48].

BAFF is a B cell growth factor that promotes B cell proliferation and leads to the generation of autoantibodies in people with RA [49]. RANKL is a protein that regulates osteoclast formation and is a key cause of bone degradation in RA [50]. Additionally, neutrophils in the synovial fluid of people with RA can display MHC class II molecules and deliver antigen to T lymphocytes [51], a role they share with macrophages and dendritic cells.Neutrophils also play a role in the inflammatory cytokine and chemokine cascades, as well as regulating immune

responses through cell-cell contacts [43].In comparison to healthy individuals, neutrophils from RA patients produce spontaneous NETs [41].

Systemic Lupus Erythematosus

SLE is a chronic systemic autoimmune disease characterised by a wide range of immunological and laboratory abnormalities as well as clinical manifestations. Lupus can damage practically any organ, although the kidney and the central nervous system are particularly vulnerable. Women are the ones who are most affected [52].

Neutrophils rapidly accumulate in sites of tissue injury, whether or not infection is present, and prevent bacteria and fungus from invading further. In patients with SLE, a lack of NET clearance has been linked to high anti-NET antibody titers and renal damage [38]. Neutrophils from SLE patients have a variety of phenotypic and functional abnormalities. Patients with SLE have an increased number of circulating apoptotic neutrophils in their peripheral blood, which correlates with disease activity and may give excess autoantigen such as dsDNA [53]. When tolerance to self-antigens is lost, autoreactive B lymphocytes are stimulated and autoantibodies are generated against nucleic acids and AMPs, which are created by infiltrating neutrophils that undergo NETosis in the skin and kidneys of patients with SLE.

The immune complexes that are produced can deposit in many tissues, causing injury and inflammation, which can lead to skin lesions, nephritis, and cardiovascular disease [54,55]. Furthermore, autoantigens that have been opsonized cause pDCs to release IFN-α, and neutrophils to form NETs [56,57]. A subpopulation of neutrophils found in SLE patients has been identified as having a high number of low-density granulocytes (LDG).

TNF and type 1 IFN were constantly produced by low-density granulocytes, and NETosis occurred spontaneously [35]. Furthermore, in SLE patients, elevated IFN-α is a crucial driving element that primes neutrophils for NETosis execution [58]. In SLE patients, not only the generation but also the degradation of NETs is affected.

Monocyte-derived macrophages (MoMa) efficiently clear NETs in normal settings, and this process is aided by the extracellular pretreatment of NETs by DNase I and C1q [59]. DNase I and C1q have previously been shown to work together to degrade chromatin [60]. NETs are transported to lysosomes by phagosomes after being consumed by macrophages. Because NET uptake by macrophages does not result in pro-inflammatory cytokine output, it is immunologically silent [59].

Anti-DNase antibodies were found in 62% of SLE patients' sera, compared to only 8 percent of healthy controls, according to Yeh et al. They also discovered a link between anti-DNase and anti-DNA antibodies in SLE patients' sera. Antibodies that recognize a conserved epitope near DNase's catalytic region protect NETs against degradation and hence may play a role in SLE pathogenesis [61].

Type-1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that causes hyperglycemia by destroying β pancreatic cells in genetically vulnerable individuals. Autoantigens are presented

when β pancreatic cells die. These autoantigens are recognized by the autoreactive T cells and also lead to the generation of specific autoantibodies for β cell antigens [62, 63, 64, 65].

Hyperglycemia causes neutrophils to create more superoxide and cytokines, including TNF-α, which causes NETosis [66, 67]. According to research, hyperglycemia may also aidNETosis. An increase in TNF-α, as seen in diabetics, leads to neutrophils to form NETs and release their intracellular contents, which include neutrophil serine proteases [68]. These proteins have a critical role in the pathogenesis of T1DM due to their function in the maturation and release of the cytokines IFN-α, IL-1β, and IL-18 as well as in the formation and activation of TLRs, which are crucial mediators of insulitis and β-pancreatic cell death [69, 70]. They also encourage neutrophil migration to inflammatory areas, generating negative feedback and contributing to autoimmune diabetes development [71].

Immunothrombosis (COVID-19)

As the SARS-CoV-2 epidemic prevails, research suggests that microthrombi and coagulopathy have a role in COVID-19 development and a wide range of clinical manifestations. Up to 10% of COVID-19 patients have shown a risk of having multiorgan failure and may require admission to an intensive care unit (ICU) for acute respiratory distress syndrome (ARDS) [72].

Immunothrombosis, the innate immune response's direct interaction of activated leukocytes with platelets and plasma coagulation factors, may have a role in the thrombotic events seen in COVID-19 individuals with coagulopathy [73].

Various diseases, including viruses, have been found to cause NET development in studies. Though advantageous to the host's defence, the collateral harm caused by the long-term creation of NETs might set off a chain reaction of inflammatory responses. Such reactions can cause tissue degradation, promote microthrombosis, and permanently harm organs in the pulmonary and cardiovascular systems, among other things [74, 75, 76]. In hospitalised COVID-19 patients, elevated blood levels of NETs have been reported, suggesting that these structures may play a role in the disease's pathophysiology and have a link to the patients' prognosis [77].

In COVID-19, pathogenic immunothrombosis may also be caused by dysregulated neutrophil extracellular trap (NET) production [75]. As a result, ARDS and death in COVID-19 associated NET-mediated inflammatory and thrombotic tissue damage and can be reduced by breaking down or blocking NETs in COVID-19 [75].

Anti-neutrophil cytoplasm antibody (ANCA)-associated systemic vasculitis (AASV)

In perivascular tissues, ANCAs are produced, leukocytoclasis occurs, and unscavenged apoptotic and necrotic neutrophils accumulate which characterises Antineutrophil Cytoplasmic Antibody (ANCA)-associated vasculitis (AAV) [78, 79].

Many ANCAs target PR3 or MPO, enzymes that are present in neutrophil azurophilic granules and on the surfaces of NETs [80]. ANCA-stimulated neutrophils are said to release NETs, which contain the autoantigens PR3 and MPO [81]. Furthermore, circulating MPO–DNA complexes have been found in patients with AASV, implying that NET formation in these multi-

system autoimmune disorders causes vascular inflammation and amplifies autoimmune responses against neutrophil components [82].

In 2009, Kessenbrock et al. conducted biopsies of AAV patients and showed NETs present in the glomeruli in the kidney of the patients [82]. Further studies indicated that neutrophil extracellular traps can also be shown in skin lesions [83, 84] and thrombi from samples taken from AAV patients [85, 86].

Adult-onset Still's disease

Adult-onset Still's disease (AOSD) is a rare multigenic systemic autoinflammatory condition that is the adult equivalent of juvenile idiopathic arthritis (JIA) [87]. Fever, arthritis, lymphadenopathy, hepatosplenomegaly, and rash are the most common clinical symptoms of AOSD [88]. Acute phase reactants such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and ferritin are raised in the majority of patients during active illness episodes [89].

Studies conducted by Hu et al. showed that in the circulation of patients with AOSD, the amounts of cell-free DNA and NET-DNA complexes were much higher than in healthy controls, and newly separated neutrophils from patients with AOSD were more susceptible to high levels of spontaneous NET release. It was also documented that NADPH oxidase inhibitors and a mitochondrial scavenger inhibited increased NET release.

NLRP3 inflammasomes were also triggered by DNA isolated from AOSD NETs. The activation of CD68+CD86+ macrophages was accelerated by NET DNA from AOSD, which significantly boosted the expression of interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α [90]. This suggested that an accelerated NET formation contributes to AOSD pathogenesis as it mediates activation of NLRP3 and proinflammatory macrophages.

Tumour and NETs

The relationship between NET development, carcinogenesis, tumour growth, and metastasis has been broadly studied, and multiple reports have shown NETs' direct influence on cancer cell proliferation through proteases or activating signals [91, 92, 93, 94]. Patients with several forms of malignancies, including lung, pancreatic, and bladder cancer, contained higher levels of NETs in their plasma than healthy controls [95].

Lewis lung cancer and Ewing sarcoma have been found to have a huge necrotic region of dead neutrophils and NET-like structures [91, 96]. Cancer-associated thrombosis in tumours has been found to be stimulated by NETs, a condition linked to a poor prognosis in patients [92,97].

Applications

NETs as antimicrobial agent

NETs have been found to have a positive impact on bacterial infection management. Antimicrobial characteristics are found in components such as cathepsin G, histones, MPO, NE, lactoferrin, gelatinase, antimicrobial peptide-LL37, proteinase 3, pentraxin 3, and peptidoglycan-binding proteins [98, 99, 100]. According to Vidal Delgado, NETs limit or destroy microorganisms such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Shigella sonnei, Salmonella typhimurium,* and *Shigella flexneri.*

In viral conditions

There is an increased neutrophil recruitment in viral illnesses such as influenza, HIV, and respiratory syncytial virus[101,102]. These viruses induce NETosis by releasing ROS species through TLR and the NETs capture, confine, and destroy viruses or impede viral replication by blocking the PKC pathway. Histones are also very crucial for viral aggregation and then to start neutralisation, which leads to reduction of viral replication significantly & also limits its activity [103,104].

Aspergillus nidulans, A. fumigatus, Candida albicans and *Cryptococcus spp*. cause NETosis by binding to β-glucan on hyphae by extracellular matrix components [10,104,105]. In vivo, NETs have been demonstrated to be significant in capturing and removing big pathogens, making them crucial for disease prevention.

In fungal conditions

Cryptococcus, A. nidulans, C. albicans and A. fumigatus spp. is the main reason for NETosis through the recognition of β-glucan present on hyphae by extracellular matrix components or by activating NOX. NETs have been shown to be essential for antifungal defence by capturing and removing big pathogens *in vivo* [105, 106, 107].

In parasitic conditions

Platelets, monocytes, and neutrophils are activated by *Plasmodium falciparum* and *Toxoplasma gondii*. The NET formation, which is reliant on the MEK–ERK pathway, prevents parasites from spreading by trapping and killing them [108,109]. Histones inhibit *Leishmania spp*. replication and have been proven to kill these pathogens when combined with other NETs-associated chemicals including NE, MPO, and collagenase.The vast bulk of NET research has been conducted in mice and *in vitro*, although the exact mechanism of NETs *in vivo* is still unknown. Extensive research is required to clearly define and understand their influence in vivo and in humans.

NETs as biomarkers

The ability to identify NETs can be used as a prediction tool for the patients with illnesses that possess a high probability of NET production, allowing clinicians to give more tailored care. There must be research to standardise and define normal and abnormal levels for NETs to be used as screening tools. This could include blood tests for cfDNA, CitH3, NE, and MPO, which are all NET-related products.

A simple nucleic acid–staining technique has been used to quantify cfDNA in serum samples from colorectal and breast cancer patients. This is in particular utilised to separately distinguish the tumour; however, measures circulating MPO/cfDNA conjugates and CitH3 for NET analysis are more specific than cfDNA alone [110]. CitH3 is more specific to NETosis, making it a worthy tool for figuring out how NET levels differ [111]. Thalin discovered that a high CitH3 plasma concentration was a significant predictor of short-term death in certain cancer patients [112] and some observational studies support the role of NETs in colorectal cancer progression. To definitively define the differing amounts of NETs and link them to harmful cancer/disease outcomes, more extensive human research is needed.

NETs as potential targets in cancer therapy

NETs have the potential to be effective cancer treatment targets. Despite the relevance of NETs in boosting cancer cells' metastatic potential, inhibiting NET production and/or activity in tumours could be beneficial [113]. However, ongoing scientific trials are yet to determine the most effective treatment for NETs [114, 115]. Gonzalez-Aparicio et al. proposed a combination of immunotherapy and medicines that block neutrophil chemoattraction and NET extrusion as a potential cancer treatment [116]. Another difficulty to consider is the paucity of signs that can anticipate a patient's response to NETs-interfering medication. H3Cit and MPO-DNA are the only available markers of NET development, and they may have predictive significance in cancer patients [117, 118]. Patient selection criteria for NET targeting medicines were developed based on the detection of NETs in tumour biopsies and the expression of G-CSF in human tumour samples although, these studies did not present any conclusive data [113, 119]. More research is needed into the semi-quantitative method for NET detection in body fluids based on the interaction of DNA with MPO [120]. According to Jorch and Kubes, a vast number of experimental and clinical investigations targeting NETs have been conducted for pathologies other than cancer, such as autoimmune and pulmonary disorders, or consequences of autoimmune conditions [121].

Further reading/Future prospective

Neutrophils play a key role in the defence strategies used by our body to combat the pathogens. Neutrophils are immune cells that are a part of the innate immunity which use a variety of pathways to destroy antigens, including the release of NETs. NETs are a web-like structure made up of antimicrobial proteins and DNA that are generated during NETosis, a specific type of programmed neutrophil cell death. In general, NETs allow neutrophils to eliminate external infections while minimizing host cell damage, but they can potentially trigger an autoimmune reaction.

Understanding the formation and function of NETosis will lead to a better understanding of various autoimmune diseases, cancer biomarkers and diseases such as malaria and diabetes. This will also pave the way for better designing the tailored medicines for tumours and cancers. As more and more researches are taking place in this field of research and development, this certainly has a lot of potential in clinical and therapeutic advancements. This has a lot of potential and promises to keep in the area of biomedical research and we are sure that this review will prove to be one of the good reference points.

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REFERENCES

[1] Hellebrekers, P., Vrisekoop, N., Koenderman, L. (2018): Neutrophil phenotypes in health and disease. European Journal of Clinical Investigation: e12943.
[2] Kobayashi, S.D., Malachowa, N., DeLeo, F.R. (2018): Neutrophils and Bacterial Immune Evasion. Journal of Innate Immunity 5(6): 432-441.
[3] Crotty, S. (2015): A brief history of T cell help to B cells. Nature reviews Immunology 3: 185-9.
[4] Lackie, J.M. (2007): The Dictionary of Cell & Molecular Biology. Elsevier
[5] Janeway, C.J., Travers, P., Walport, M. (2001): Immunobiology: The Immune System in Health and Disease. 5th ed. New York: Garland Science.
[6] Urban, C.F., Ermert, D., Schmid, M., Abu-Abed, U., Goosmann, C, Nacken, W., Brinkmann, V., Jungblut, P.R., Zychlinsky, A. (2009): Neutrophil Extracellular Traps Contain Calprotectin, a Cytosolic Protein Complex Involved in Host Defense against Candida albicans. PLoS Pathogens 10: e1000639.
[7] Takei H, Araki A, Watanabe H, Ichinose A, Sendo F (1996): Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis. Journal of Leukocyte Biology 2: 229-240.
[8] Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., Zychlinsky, A. (2004): Neutrophil Extracellular Traps Kill Bacteria. Science 5663: 1532-1535.
[9] Brinkmann, V., Zychlinsky, A. (2007): Beneficial suicide: why neutrophils die to make NETs. Nature Reviews Microbiology 8: 577-582.
[10] Amulic, B., Knackstedt, S.L., Abu Abed, U., Deigendesch, N., Harbort, C.J, et al. (2017) Cell-Cycle Proteins Control Production of Neutrophil Extracellular Traps. Developmental Cell 4: 449-462.e5.
[11] Nirmala, J.G., Lopus, M. (2020): Cell death mechanisms in eukaryotes. Cell Biology and Toxicology 2: 145-164.
[12] Nauseef, W.M., Borregaard, N. (2014): Neutrophils at work. Nature Immunology 7: 602-611.
[13] Pires, R.H., Felix, S.B., Delcea, M. (2016): The architecture of neutrophil extracellular traps investigated by atomic force microscopy. Nanoscale 29: 14193-14202.
[14] Clark, S.R., Ma, A.C., Tavener, S.A., McDonald, B., Goodarzi, Z, Kelly, M.M., Patel, M.M., Chakrabarti, S., McAvoy, E., Sinclair, G.D., (2007) Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nature Medicine 4: 463-469.
[15] Metzler, K.D., Goosmann, C., Lubojemska, A., Zychlinsky, A., Papayannopoulos, V. (2014): A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. Cell reports 3: 883-96.
[16] Papayannopoulos, V., Metzler, K.D., Hakkim, A., Zychlinsky, A., (2010): Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. Journal of Cell Biology 3: 677-691.

[17] Repnik, U., Hafner Česen, M., Turk, B. (2014): Lysosomal membrane permeabilization in cell death: Concepts and challenges. Mitochondrion: 49-57.
[18] Fuchs, T.A., Abed, U., Goosmann, C., Hurwitz, R., Schulze I, Wahn, V., Weinrauch, Y., Brinkmann, V., Zychlinsky, A. (2007): Novel cell death program leads to neutrophil extracellular traps. Journal of Cell Biology 2: 231-241.
[19] Bianchi, M., Hakkim, A., Brinkmann, V., Siler, U., Seger, R.A, Zychlinsky, A., Reichenbach, J., (2009): Restoration of NET formation by gene therapy in CGD controls aspergillosis. Blood 13: 2619-2622.
[20] Lim, M.B.H., Kuiper, J.W.P., Katchky, A., Goldberg, H., Glogauer, M. (2011): Rac2 is required for the formation of neutrophil extracellular traps. Journal of Leukocyte Biology 4: 771-776.
[21] Kaplan, M.J., Radic, M. (2012): Neutrophil Extracellular Traps: Double-Edged Swords of Innate Immunity. The Journal of Immunology 6: 2689-2695.
[22] Remijsen, Q., Berghe, T., Vanden, Wirawan, E., Asselbergh, B., Parthoens, E., Rycke, R.D., Noppen, S., Delforge, M., Willems, J., Vandenabeele, B. (2011): Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. Cell Research 2: 290-304.
[23] Parker, H., Dragunow, M., Hampton, M.B., Kettle, A.J., Winterbourn, C.C. (2012): Requirements for NADPH oxidase and myeloperoxidase in neutrophil extracellular trap formation differ depending on the stimulus. Journal of Leukocyte Biology 4: 841-849.
[24] Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., et al. (2009): Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. Journal of Cell Biology 2: 205-213.
[25] Wang, Y., Wysocka, J., Sayegh, J., Lee, Y.H., Perlin, J.R., eLeonelli, L., Sonbuchner, L.S., Mcdonald, C.H., Cook, R.G., Dou, Y., Roeder, R.G., Clarke, S., Stallcup, M.R., Allis, D., Coonrod, S.A. (2004): Human PAD4 Regulates Histone Arginine Methylation Levels via Demethylimination. Science 5694: 279-283.
[26] 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 7: e22043.
[27] 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. Journal of Experimental Medicine 9: 1853-1862.
[28] Yousefi, S., Simon, D., Stojkov, D., Karsonova, A., Karaulov, A., Simon, H.U. (2020): *In vivo* evidence for extracellular DNA trap formation. Cell Death & Disease 4: 300.
[29] Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., et al. (2018): Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death & Differentiation 3: 486-541.
[30] Yousefi, S., Gold, J.A., Andina, N., Lee, J.J., Kelly, A.M., Kozlowsky, E., Schmid, I., Straumann, A., Reichenbach, J., Gleich, G.J., Simon, H.U. (2008): Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. Nature Medicine 9: 949-953.
[31] Yousefi, S., Mihalache, C,. Kozlowski, E., Schmid, I., Simon, H.U. (2009): Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. Cell Death & Differentiation 11: 1438-1444.
[32] Morshed, M., Hlushchuk, R., Simon, D., Walls, A.F., Obata-Ninomiya, K., Karasuyama, H., Djonov, V., Eggel, A., Kaufmann, T., Simon, H.U. (2014): NADPH oxidase-independent formation of extracellular DNA traps by basophils. Journal of immunology (Baltimore, Md : 1950) 11: 5314-23.
[33] Ingelsson, B., Söderberg, D., Strid, T., Söderberg, A., Bergh, A.C., Loitto, V., Lotfi, K., Segelmark, M., Spyrou, G., Rosén, A. (2018): Lymphocytes eject interferogenic mitochondrial DNA webs in response to CpG and non-CpG oligodeoxynucleotides of class C. Proceedings of the National Academy of Sciences 3.
[34] Lood, C., Blanco, L.P., Purmalek, M.M., Carmona-Rivera, C., De Ravin, S.S., Smith, C. K., Malech, H.L., Ledbetter, J.A., Elkon, K.B., Kaplan, M.J. (2016): Neutrophil extracellular traps enriched in

oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nature Medicine 2: 146-153.

[35] Kaplan, M.J. (2011): Neutrophils in the pathogenesis and manifestations of SLE. Nature Reviews Rheumatology 12: 691-699.

[36] White, M.J., McArthur, K., Metcalf, D., Lane, R.M., Cambier, J.C., Herold, M.J., van Delft, M.J., Bedoui, S., Lessene, G., Ritchie, M.E., Huang, D.S.C., Kile, B.T. (2014): Apoptotic Caspases Suppress mtDNA-Induced STING-Mediated Type I IFN Production. Cell 7: 1549-1562.

[37] Martinelli, S., Urosevic, M., Daryadel, A., Oberholzer, P.A., Baumann, C,. Fey, M.F., Dummer, R., Simon, H.U., Yousefi, S. (2004): Induction of Genes Mediating Interferon-dependent Extracellular Trap Formation during Neutrophil Differentiation. Journal of Biological Chemistry 42: 44123-44132.

[38] Hakkim, A., Fürnrohr, B.G., Amann, K., Laube, B., Abed, U.A., et al. (2010): Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proceedings of the National Academy of Sciences 21: 9813-9818.

[39] Janko, C., Schorn, C., Grossmayer, G.E., Frey, B., Herrmann, M., et al. (2008): Inflammatory clearance of apoptotic remnants in systemic lupus erythematosus (SLE). Autoimmunity Reviews 1: 9-12.

[40] Zhao, Y., Marion, T.N., Wang, Q. (2019): Multifaceted Roles of Neutrophils in Autoimmune Diseases. Journal of Immunology Research: 1-2.

[41] Pérez-Sánchez, C., Ruiz-Limón, P., Aguirre, M.A., Jiménez-Gómez, Y., Arias-de la Rosa, I., et al. (2017): Diagnostic potential of NETosis-derived products for disease activity, atherosclerosis and therapeutic effectiveness in Rheumatoid Arthritis patients. Journal of Autoimmunity: 31-40.

[42] Eggleton, P., Wang, L., Penhallow, J., Crawford, N., Brown, K.A. (1995): Differences in oxidative response of subpopulations of neutrophils from healthy subjects and patients with rheumatoid arthritis. Annals of the Rheumatic Diseases 11: 916-923.

[43] Wright, H.L., Moots, R.J., Edwards, S.W. (2014): The multifactorial role of neutrophils in rheumatoid arthritis. Nature Reviews Rheumatology 10: 593-601.

[44] Fossati, G., Moots, R.J., Bucknall, R.C., Edwards, S.W. (2002): Differential role of neutrophil Fc? receptor IIIB (CD16) in phagocytosis, bacterial killing, and responses to immune complexes. Arthritis & Rheumatism 5: 1351-1361.

[45] Assi, L.K., Wong, S.H., Ludwig, A., Raza, K., Gordon, C., et al. (2007): Tumor necrosis factor α activates release of B lymphocyte stimulator by neutrophils infiltrating the rheumatoid joint. Arthritis & Rheumatism 6: 1776-1786.

[46] Tanaka, S. (2018): Emerging anti-osteoclast therapy for rheumatoid arthritis. Journal of Orthopaedic Science 5: 717-721.

[47] Lupia, E., Montrucchio, G., Battaglia, E., Modena, V., Camussi, G. (1996): Role of tumor necrosis factor-α and platelet-activating factor in neoangiogenesis induced by synovial fluids of patients with rheumatoid arthritis. European Journal of Immunology 8: 1690-1694.

[48] Wright, H.L., Chikura, B., Bucknall, R.C., Moots, R.J., Edwards, S.W. (2011): Changes in expression of membrane TNF, NF-κB activation and neutrophil apoptosis during active and resolved inflammation. Annals of the Rheumatic Diseases 3: 537-543.

[49] Wei, F., Chang, Y., Wei, W. (2015): The role of BAFF in the progression of rheumatoid arthritis. Cytokine 2: 537-544.

[50] Chakravarti, A., Raquil, M.A., Tessier, P., Poubelle, P.E. (2009): Surface RANKL of Toll-like receptor 4–stimulated human neutrophils activates osteoclastic bone resorption. Blood 8: 1633-1644.

[51] Cross, A., Bucknall, R.C., Cassatella, M.A., Edwards, S.W., Moots, R.J. (2003): Synovial fluid neutrophils transcribe and express class II major histocompatibility complex molecules in rheumatoid arthritis. Arthritis & Rheumatism 10: 2796-2806.

[52] Borchers, A.T., Naguwa, S.M., Shoenfeld, Y., Gershwin, M.E. (2010): The geoepidemiology of systemic lupus erythematosus. Autoimmunity Reviews 5: A277-A287.

[53] Courtney, P.A., Crockard, A.D., Williamson, K., Irvine, A.E., Kennedy, R.J., et al. (1999): Increased apoptotic peripheral blood neutrophils in systemic lupus erythematosus: relations with disease activity, antibodies to double stranded DNA, and neutropenia. Annals of the Rheumatic Diseases 5: 309-314.

[54] Lande, R., Ganguly, D., Facchinetti, V., Frasca, L., Conrad, C., et al. (2011): Neutrophils Activate Plasmacytoid Dendritic Cells by Releasing Self-DNA–Peptide Complexes in Systemic Lupus Erythematosus. Science Translational Medicine 3(73): 73ra19-73ra19.

[55] Smith, C.K., Kaplan, M.J. (2015): The role of neutrophils in the pathogenesis of systemic lupus erythematosus. Current Opinion in Rheumatology 5: 448-453.

[56] Blanco, P., Palucka, A.K., Gill, M., Pascual, V., Banchereau, J. (2001): Induction of Dendritic Cell Differentiation by IFN-α in Systemic Lupus Erythematosus. Science 5546: 1540-1543.

[57] Mahajan, A., Herrmann, M., Muñoz, L.E. (2016): Clearance Deficiency and Cell Death Pathways: A Model for the Pathogenesis of SLE. Frontiers in Immunology.

[58] Niewold, T.B., Clark, D.N., Salloum, R., Poole, B.D. (2010): Interferon Alpha in Systemic Lupus Erythematosus. Journal of Biomedicine and Biotechnology: 1-8.

[59] Farrera, C., Fadeel, B. (2013): Macrophage Clearance of Neutrophil Extracellular Traps Is a Silent Process. The Journal of Immunology 5: 2647-2656.

[60] Gaipl, U.S., Beyer, T.D., Heyder, P., Kuenkele, S., Böttcher, A., Voll, R.E., Kalden, J.R., Hermann, M. (2004) Cooperation between C1q and DNase I in the clearance of necrotic cell-derived chromatin. Arthritis & Rheumatism 2: 640-649.

[61] Yeh, T.M., Chang, H.C., Liang, C.C., Wu, J.J., Liu, M.F. (2003): Deoxyribonuclease-Inhibitory antibodies in systemic lupus erythematosus. Journal of Biomedical Science 5: 544-551.

[62] Atkinson MA, Eisenbarth GS, Michels AW (2014) Type 1 diabetes. The Lancet 9911: 69-82.

[63] Gan, M.J., Albanese-O'Neill, A., Haller, M.J. (2012): Type 1 Diabetes: Current Concepts in Epidemiology, Pathophysiology, Clinical Care, and Research. Current Problems in Pediatric and Adolescent Health Care 10: 269-291.

[64] Skyler, J.S., Sosenko, J.M. (2013): The Evolution of Type 1 Diabetes. JAMA 23: 2491.

[65] Van Belle, T.L., Coppieters, K.T., Von Herrath, M.G. (2011): Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. Physiological Reviews 1: 79-118.

[66] Hanses, F., Park, S., Rich, J., Lee, J.C. (2011): Reduced Neutrophil Apoptosis in Diabetic Mice during Staphylococcal Infection Leads to Prolonged Tnfα Production and Reduced Neutrophil Clearance. PLoS ONE 8: e23633.

[67] Alexandraki, K.I., Piperi, C., Ziakas, P.D., Apostolopoulos, N. V., Makrilakis, K, et al. (2008): Cytokine Secretion in Long-standing Diabetes Mellitus Type 1 and 2: Associations with Low-grade Systemic Inflammation. Journal of Clinical Immunology 4: 314-321.

[68] Valle, A., Giamporcaro, G.M., Scavini, M., Stabilini, A., Grogan, P., et al. (2013): Reduction of Circulating Neutrophils Precedes and Accompanies Type 1 Diabetes. Diabetes 6: 2072-2077.

[69] 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. Annals of the New York Academy of Sciences 1: 16-35.

[70] Grieco, F.A., Vendrame, F., Spagnuolo, I., Dotta, F. (2011): Innate immunity and the pathogenesis of type 1 diabetes. Seminars in Immunopathology 1: 57-66.

[71] Wang, Y., Xiao, Y., Zhong, L., Ye, D., Zhang, J., Tu, Y., Bornstein, S.R., Zhou, Z., Lam, K. S. L., Xu, A. (2014): Increased Neutrophil Elastase and Proteinase 3 and Augmented NETosis Are Closely Associated With β-Cell Autoimmunity in Patients With Type 1 Diabetes. Diabetes 12: 4239-4248.

[72] Emanuel, E.J., Persad, G., Upshur, R., Thome, B., Parker, M., et al. (2020): Fair Allocation of Scarce Medical Resources in the Time of Covid-19. New England Journal of Medicine 21: 2049-2055.

[73] Manne, B.K., Denorme, F., Middleton, E.A., Portier, I., Rowley, J.W., Stubben, C., Petrey, A.C., Tolley, N.D., Guo, L., Cody, M., Weyrich, A.S., Yost, C.C., Rondina, M.T., Campbell, R.A. (2020): Platelet gene expression and function in patients with COVID-19. Blood 11: 1317-1329.

[74] Rosales, C. (2020): Neutrophils at the crossroads of innate and adaptive immunity. Journal of Leukocyte Biology 1: 377-396.
[75] Barnes, B. J., Adrover, J. M., Baxter-Stoltzfus, A., Borczuk, A., Cools-Lartigue, J., Crawford, J. M., ... Egeblad, M. (2020). Targeting potential drivers of COVID-19: Neutrophil extracellular traps. Journal of Experimental Medicine, 217(6).
[76] Zuo, Y., Yalavarthi, S., Shi, H., Gockman, K., Zuo, M., Madison, J.A., Blair, C., Weber, A., Barnes, B.J., Egeblad, M., Woods, R.J., Kanthi, Y., Knight, J.S. (2020): Neutrophil extracellular traps in COVID-19. JCI Insight.
[77] Brinkmann, V. (2018): Neutrophil Extracellular Traps in the Second Decade. Journal of Innate Immunity 5-6: 414-421.
[78] Masuda, S., Nonokawa, M., Futamata, E., Nishibata, Y., Iwasaki, S., Tsuji, T., Hatanaka, Yç, Nakazawa, D., Tanaka, S., Tomaru, U., Kawakami, T., Atsumi, T., Ishizu, A. (2019): Formation and Disordered Degradation of Neutrophil Extracellular Traps in Necrotizing Lesions of Anti-Neutrophil Cytoplasmic Antibody–Associated Vasculitis. The American Journal of Pathology 4: 839-846.
[79] Franssen, C.F., Huitema, M.G., Kobold, A.C.M., Oost-Kort, W.W., Limburg, P.C., Tiebosch, A., Stegeman, C.A., Kallenberg, C.G.M., Tervaert, J.W.C, (1999) In Vitro Neutrophil Activation by Antibodies to Proteinase 3 and Myeloperoxidase from Patients with Crescentic Glomerulonephritis. Journal of the American Society of Nephrology 7: 1506-1515.
[80] Söderberg, D., Kurz, T., Motamedi, A., Hellmark, T., Eriksson, P., Segelmark, M. (2015): Increased levels of neutrophil extracellular trap remnants in the circulation of patients with small vessel vasculitis, but an inverse correlation to anti-neutrophil cytoplasmic antibodies during remission. Rheumatology 11: 2085-2094.
[81] Ramos-Kichik, V., Mondragón-Flores, R., Mondragón-Castelán, M., Gonzalez-Pozos, S., Muñiz-Hernandez, S., et al. (2009): Neutrophil extracellular traps are induced by Mycobacterium tuberculosis. Tuberculosis 1: 29-37.
[82] Kessenbrock, K., Krumbholz, M., Schönermarck, U., Back, W., Gross, W.L, Werb, Z., Gröne, H.J., Brinkmann, V., Jenne, D.E. (2009): Netting neutrophils in autoimmune small-vessel vasculitis. Nature Medicine 6: 623-625.
[83] Abreu-Velez, A.M., Smith, J.G., Howard, M.S. (2009): Presence of neutrophil extracellular traps and antineutrophil cytoplasmic antibodies associated with vasculitides. North American journal of medical sciences 6: 309-13.
[84] Sangaletti, S., Tripodo, C., Chiodoni, C., Guarnotta, C., Cappetti, B., Casalini, P., Piconese, S., Parenza, M., Guiducci, C., Vitali, C., Colombo, M.P. (2012): Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. Blood 15: 3007-3018.
[85] Nakazawa, D., Tomaru, U., Yamamoto, C., Jodo, S., Ishizu, A. (2012): Abundant neutrophil extracellular traps in thrombus of patient with microscopic polyangiitis. Frontiers in Immunology.3:333.
[86] Imamoto, T., Nakazawa, D., Shida, H., Suzuki, A., Otsuka, N., Tomaru, U., Ishizu, A. (2014): Possible linkage between microscopic polyangiitis and thrombosis via neutrophil extracellular traps. Clinical and experimental rheumatology, 32(1): 149-150.
[87] Seo, J. Y., Suh, C. H., Jung, J. Y., Kim, A. R., Yang, J. W., Kim, H. A. (2017): The neutrophil-to-lymphocyte ratio could be a good diagnostic marker and predictor of relapse in patients with adult-onset Still's disease: A STROBE-compliant retrospective observational analysis. Medicine, 96(29).
[88] Giacomelli, R., Ruscitti, P., Shoenfeld, Y. (2018): A comprehensive review on adult onset Still's disease. Journal of Autoimmunity: 24-36.
[89] Lebrun, D., Mestrallet, S., Dehoux, M., Golmard, J.L., Granger, B., Georgin-Lavialle, S., Arnaud, L., Grateau, T., Pouchot, J., Fautrel, B. (2018): Validation of the Fautrel classification criteria for adult-onset Still's disease. Seminars in Arthritis and Rheumatism 4: 578-585.
[90] Hu, Q., Shi, H., Zeng, T., Liu, H., Su, Y., Cheng, X., Ye, J., Yin, Y., Liu, M., Zheng, H., Wu, X., Chi, H., Zhou, Z., Jia, J., Sun, Y., Teng, J., Yang, C. (2019): Increased neutrophil extracellular traps

activate NLRP3 and inflammatory macrophages in adult-onset Still's disease. Arthritis Research & Therapy 1: 9.
[91] Berger-Achituv, S., Brinkmann, V., Abed, U.A., Kühn, L.I., Ben-Ezra, J., Elhasid, R., Zychlinsky, A. (2013): A proposed role for neutrophil extracellular traps in cancer immunoediting. Frontiers in Immunology.
[92] Cools-Lartigue, J., Spicer, J., Najmeh, S., Ferri, L. (2014): Neutrophil extracellular traps in cancer progression. Cellular and Molecular Life Sciences 21: 4179-4194.
[93] Homa-Mlak, I., Majdan, A., Mlak, R., Małecka-Massalska, T. (2016): Metastatic potential of NET in neoplastic disease. PostępyHigienyiMedycynyDoświadczalnej: 887-895.
[94] Sangaletti, S., Tripodo, C., Vitali, C., Portararo, P., Guarnotta, C., Casalini, P., Cappetti, B., Miotti, S., Pinciroli, P., Fuligni, F., Fais, F., Piccaluga, P.P., Colombo, M.P. (2014): Defective Stromal Remodeling and Neutrophil Extracellular Traps in Lymphoid Tissues Favor the Transition from Autoimmunity to Lymphoma. Cancer Discovery 1: 110-129.
[95] Oklu, R., Sheth, R.A., Wong, K.H.K., Jahromi, A.H., Albadawi, H. (2017): Neutrophil extracellular traps are increased in cancer patients but does not associate with venous thrombosis. Cardiovascular Diagnosis and Therapy S3: S140-S149.
[96] Ho-Tin-Noé, B., Carbo, C., Demers, M., Cifuni, S.M., Goerge, T., Wagner, D.D. (2009): Innate Immune Cells Induce Hemorrhage in Tumors during Thrombocytopenia. The American Journal of Pathology 4: 1699-1708.
[97] Demers, M., Wagner, D. (2014): NETosis: A New Factor in Tumor Progression and Cancer-Associated Thrombosis. Seminars in Thrombosis and Hemostasis 03: 277-283.
[98] Brinkmann, V., Zychlinsky, A. (2012): Neutrophil extracellular traps: Is immunity the second function of chromatin? Journal of Cell Biology 5: 773-783.
[99] Neumann, A., Berends, E.T.M., Nerlich, A., Molhoek, E.M., Gallo, R.L., Meerloo, T., Nizet, V., Naim, H.Y., von Köckritz-Blickwede, M. (2014): The antimicrobial peptide LL-37 facilitates the formation of neutrophil extracellular traps. Biochemical Journal 1: 3-11.
[100] Burgener, S.S., Schroder, K. (2020): Neutrophil Extracellular Traps in Host Defense. Cold Spring Harbor Perspectives in Biology 7: a037028.
[101] Borchers, A.T., Chang, C., Gershwin, M.E., Gershwin, L.J. (2013): Respiratory Syncytial Virus—A Comprehensive Review. Clinical Reviews in Allergy & Immunology 3: 331-379.
[102] Saitoh, T., Komano, J., Saitoh, Y., Misawa, T., Takahama, M., Kozaki, T., Uehata, T., Iwasaki, H., Omori, H., Yamaoka, S., Yamamoto, N., Akira, S. (2012): Neutrophil Extracellular Traps Mediate a Host Defense Response to Human Immunodeficiency Virus-1. Cell Host & Microbe 1: 109-116.
[103] Gwyer Findlay, E., Currie, S.M., Davidson, D.J. (2013): Cationic Host Defence Peptides: Potential as Antiviral Therapeutics. BioDrugs 5: 479-493.
[104] McCormick, A., Heesemann, L., Wagener, J., Marcos, V., Hartl, D., Loeffler, J., Heesemann, J., Ebel, F. (2010): NETs formed by human neutrophils inhibit growth of the pathogenic mold Aspergillus fumigatus. Microbes and Infection 12-13: 928-936.
[105] Branzk, N., Lubojemska, A., Hardison, S.E., Wang, Q., Gutierrez, M.G., Brown, G.D., Papayannopoulos, V. (2014): Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nature Immunology 11: 1017-1025.
[106] Bruns, S., Kniemeyer, O., Hasenberg, M., Aimanianda, V., Nietzsche, S., Thywißen, A., Jeron, A., Latge, J.P., Bralhage, A.A., Gunzer, M. (2010): Production of Extracellular Traps against Aspergillus fumigatus In Vitro and in Infected Lung Tissue Is Dependent on Invading Neutrophils and Influenced by Hydrophobin RodA. PLoS Pathogens 4: e1000873.
[107] Carroll, G.M., Burns, G.L., Petit, J.A., Walker, M.M., Mathe, A., et al. (2020): Does postoperative inflammation or sepsis generate neutrophil extracellular traps that influence colorectal cancer progression? A systematic review. Surgery Open Science 2: 57-69.
[108] Baker, V.S., Imade, G.E., Molta, N.B., Tawde, P., Pam, S.D,, Obadofin, M.O., Sagay, S.A., Egah, D.Z., Iya, D., Afolabi, B.B., Baker, M., Ford, K., Roux, K.H., CS Keller III, T. (2008): Cytokine-

associated neutrophil extracellular traps and antinuclear antibodies in Plasmodium falciparum infected children under six years of age. Malaria Journal 1: 41.

[109] 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. Infection and immunity, 80(2): 768-777.

[110] Zhu, L., Liu, L., Zhang, Y., Pu, L., Liu, J., Li, X., Chen, Z., Hao, Y., Wang, B., Han, J., Li, G., Liang, S., Xiong, H., Zheng, H., Li, A., Xu, J., Zeng, H. (2018): High level of neutrophil extracellular traps correlates with poor prognosis of severe influenza A infection. The Journal of infectious diseases, 217(3): 428-437.

[111] Li, R.H.L., Johnson, L.R., Kohen, C., Tablin, F. (2018): A novel approach to identifying and quantifying neutrophil extracellular trap formation in septic dogs using immunofluorescence microscopy. BMC Veterinary Research 1: 210.

[112] Thålin, C., Hisada, Y., Lundström, S., Mackman, N., Wallén, H. (2019): Neutrophil Extracellular Traps. Arteriosclerosis, Thrombosis, and Vascular Biology 9: 1724-1738.

[113] Park, J., Wysocki, R. W., Amoozgar, Z., Maiorino, L., Fein, M. R., Jorns, J., Schott, A.F., Kınugasa-Katayama, Y., Lee, Y., Heewon, N., Nakasone, S., Hearn, S.A., Kuttner, V., Qiu, J., Almedia, A.S., Perurena, R., Kessenbrock, K., Goldberg, M.S., Egeblad, M. (2016): Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Science translational medicine, 8(361), 361ra138-361ra138.

[114] Saffarzadeh, M. (2019): Neutrophil Extracellular Traps as a Drug Target to Counteract Chronic and Acute Inflammation. Current Pharmaceutical Biotechnology 15: 1196-1202.

[115] Wu, L., Saxena, S., Awaji, M., Singh, R. K. (2019): Tumor-associated neutrophils in cancer: going pro. Cancers, 11(4): 564.

[116] Gonzalez-Aparicio, M., Alfaro, C. (2019): Influence of Interleukin-8 and Neutrophil Extracellular Trap (NET) Formation in the Tumor Microenvironment: Is There a Pathogenic Role? Journal of Immunology Research: 1-7.

[117] Tohme, S., Yazdani, H.O., Al-Khafaji, A.B., Chidi, A.P., Loughran, P, Mowen, K., Wang, Y., Simmons, R.L., Huang, H., Tsung, A. (2016): Neutrophil Extracellular Traps Promote the Development and Progression of Liver Metastases after Surgical Stress. Cancer Research 6: 1367-1380.

[118] Thålin, C., Lundström, S., Seignez, C., Daleskog, M., Lundström, A., Henriksson, P., Helleday, T., Philipson, M., Wallen, H., Demers, M. (2018): Citrullinated histone H3 as a novel prognostic blood marker in patients with advanced cancer. PLOS ONE 1: e0191231.

[119] Hollmén, M, Karaman, S,. Schwager, S., Lisibach, A., Christiansen, A.J., Maksimow, M., Varga, Z., Jalkanen, S., Detmar, M. (2016): G-CSF regulates macrophage phenotype and associates with poor overall survival in human triple-negative breast cancer. OncoImmunology 3: e1115177.

[120] Koenen, R.R. (2014): Neutrophil Extracellular Traps as Therapeutic Targets for Inflammatory Disease. American Journal of Pharmacology and Toxicology 4: 200-202.

[121] Jorch, S.K., Kubes, P (2017): An emerging role for neutrophil extracellular traps in noninfectious disease. Nature Medicine 3: 279-287.