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REVIEW
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A Well-Intentioned Enemy in Autoimmune and Autoinflammatory Diseases: NETosis

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ABSTRACT

Neutrophils are an essential member of the innate immune system derived from the myeloid stem cell series and develop in the bone marrow. The action of neutrophils defined in immune response includes phagocytosis, degranulation, cytokine production, and neutrophil extracellular traps. The success of the host immune defense depends on effective neutrophil activation. Recent studies have shown that neutrophils that have completed their task in the field of inflammation rejoin circulation. Uncontrolled inflammatory response and dysregulated immune responses to the host are important factors in the development of acute and chronic diseases. Neutrophils are the first cells to be drawn into the field at the time of inflammation. They have developed response strategies that produce proinflammatory cytokines and are known as neutrophil extracellular traps since they create mesh-like structures with their DNA contents into the external environment and release their granular proteins in this way. This article summarizes numerous recent studies and reviews the role of neutrophil extracellular traps in autoimmune and autoinflammatory diseases in the hope, that this will lead to the development of more effective treatments. In addition, in this review, the role of neutrophil extracellular trap formation in some pediatric autoimmune diseases is emphasized.

Keywords: NETosis, autoimmune, autoinflammatory

INTRODUCTION

Neutrophils are an essential member of the innate immune system derived from the myeloid stem cell series and develop in the bone marrow. Seventy percent of "circulating" leukocytes are neutrophils and have a lifespan ranging from 8 hours to 5 days.[1,2] The amount of neutrophils in the blood is determined by bone marrow granulopoiesis, the pool in the vascular bed, extravasation into tissues, and macrophage efferocytosis.[3,4] They are precursor cells in tissue repair, particularly in pathogen clearance because they are the first cells attracted to areas of infection and tissue damage. The mechanisms of action of neutrophils defined in recent years include phagocytosis, degranulation, cytokine production, and neutrophil extracellular traps (NETs).[5,6] Most blood neutrophils are referred to as high-density neutrophils (HDNs), which have large granules. On the other hand, low-density neutrophils (LDNs) have also been defined recently.[7] Depending on their physiological and pathophysiological roles, LDNs have been reported to originate from bone marrow precursors,[8] whereas HDNs are degranulated or have enlarged cytoplasms.[9] According to their action mechanism on tumor cells, neutrophils can be classified as N1 and N2.[10] Another classification can be made according to their surface molecules. Neutrophils that express intracellular adhesion molecule 1 and chemokine receptor CXCR1 are classified separately because they show different migration strategies[11] and angiogenic activity.[12,13] It is known that microbial content and certain cytokines are inducing factors in bone marrow granulosis, and this determines the circulation of granulocytes, thus that of neutrophils in the blood.[14,15] The success of the host immune defense depends on effective neutrophil activation. One of the activation

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mechanisms is the triggering of damage-associated molecular patterns (DAMPs), which are chemicals released from damaged cells into the environment or proteolytic modifications that cause tissue damage. Extravasation to damaged tissue is sometimes referred to as neutrophil fusion, a complex process that begins with the recognition of DAMPs by toll-like receptors (TLRs)[16] and inflammasome, that is, pattern recognition receptors and continues with the binding of numerous lipid mediators and adhesion molecules[17,18] to P-selectin glycoprotein ligand 1 and L-selectins on neutrophils.[19,20] Neutrophil, which reaches the area of inflammation; nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and reactive oxygen radicals[21,22] and degranulation molecules[23] (defensins and lytic proteases[24]) carry out phagocytosis.[25,26] Uncontrolled inflammatory response and dysregulated immune responses to the host are important factors in the development of acute and chronic diseases. Neutrophils are the first cells to be drawn into the field at the time of inflammation. They have developed response strategies that produce proinflammatory cytokines and are known as NETs since they create mesh-like structures with their DNA contents into the external environment and release their granular proteins in this way. The toxic effects of NETs can cause tissue damage, development of autoimmune processes, vascular damage, and organ and tissue fibrosis. The results of various studies on this subject support the idea that an imbalance during the control of an infectious agent can cause serious harmful effects, contributing to the course and severity of diseases.

NEUTROPHIL EXTRACELLULAR TRAPS (NETOSIS)

In 2004, Arturo Zychlinsky et al were the first to determine that neutrophils had a new pathogen-killing mechanism and named the entity facilitating this mechanism as NETs. The actual content of NETs is chromatin, which is concentrated in extracellular space. This structure is coated with granular, nuclear, and cytoplasmic proteins, released out of the cell, which ensures the immobilization and killing of pathogens.[6] In this way, NETs can kill Gram+ and Gram– bacteria and fungi.[27] Following this discovery, the scientific world began to explore factors, signaling pathways, and different mechanisms playing a role in the formation of NETs and their structural components. Thus, the importance of NETs in both human health and diseases was gradually better understood. In particular, the roles of NETs in tissue necrosis and inflammation have been emphasized.[28] These attempts have mainly served to clarify that NET formations are related to infectious diseases and conditions with sterile tissue damage such as thrombosis, tissue necrosis, autoinflammation, and autoimmunity.[29] The roles of NET formation in appendicitis,[2] sepsis,[30] and preeclampsia[31] have been demonstrated. Neutrophil extracellular trap formation involves DNA, histones, and polymorphonuclear granular proteins.[6] This structure is an effector mechanism of the immune response and plays a role in pathogen elimination. Neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, lactoferrin, pentaxin,[17] gelatinase, proteinase,[17] and peptidoglycan-binding proteins are involved in the microbicidal potential of NETs.[32,33] Neutrophil elastase and cathepsin are serine proteases activating cytokines that trigger the formation of NETs, such as interleukin (IL)-1 and IL-36.[34] When NET formation emerges, significant changes occur in the cell. The nuclear membrane integrity of the cell is impaired, and cytoplasmic chromatin condensation and plasma membrane explosion are also observed.[6] In this scenario, NETs can also cause neutrophils to die during the cell-killing mechanism. However, this mode of death is very different from the apoptosis and necrosis of cells.[35] Therefore, the term NETosis has been used to refer to the form of cell death in response to neutrophil apoptosis without cell death at the end of each NET formation. NETosis can also describe the specific signal pathway of NET formation. There is an increasing number of studies on the determination of extracellular chromatin and protein determined using various methods.[36,37] NETosis have 2 types, vital and suicidal, according to whether the cell integrity of neutrophils is disturbed or not (Figure 1). This form of death, called suicidal NETosis, is associated with NET. However, cell viability and functions are still preserved in the mechanism called vital NETosis.[38] In fact, NETosis types are shaped by the molecular stimulation of neutrophils.[39] In recent proteomics analyses, NETs induced by phorbol 12 myristate 13 acetate (PMA), calcium ionophore A23187, or lipopolysaccharide (LPS) of *Escherichia coli* have been shown to have different protein compositions, post-translational modifications, and properties.[40] The formation of suicidal NET is due to increased intracellular reactive oxygen radicals that result in the nuclear translocation of NEs. Causing the degradation of histones by translocation to the nucleus, NE triggers chromatin condensation.[35,36] Myeloperoxidase also leads to large chromatin relaxation by synergistic action with NE.[36-41] Proteolysis by NE not only affects histone modification but also forms NETs. Histones hypercitrulinated by peptidyl arginine deaminase 4 (PAD4) cause the destabilization of the nucleosome and chromatin concentration. High levels of PAD4 are expressed on the surfaces of neutrophils. In particular, the PAD4 enzyme is responsible for the hypercitrullinylation of histone H3 and H4, chromatin condensation, and the formation of NETs.[42] Pro-inflammatory cytokines, such as IL-1β, tumor necrosis factor (TNF)-α, and IL-8 are strong inducers of reactive oxygen species (ROS) production in neutrophils, as well as NET formation.[43] Calcium mobilization and protein kinase C (PKC) isoforms have been shown to stimulate NETosis. This coordinated balance is achieved by PKCα inhibiting histone deamination and, in turn, PKCζ leading to the activation and histone citrullination of PAD4.[44] In addition, ROS activates mitogen-activated protein kinase p38 and p38 regulated/activated kinase (PRAK) is regulated by down-regulation. In this way, PMA-induced NET formation is triggered.[45] p38 regulated/activated kinase is an oxidative stress sensor, and p38-like molecules provide the balance between the apoptosis and NETosis of neutrophils.

Mechanism of Suicidal NETosis

In conventional suicidal NETosis, IgG-Fc receptor interaction occurs when TLRs, complement factors, or cytokines trigger neutrophils. These receptors modulate the activating signaling pathway downstream within the cell. Calcium (Ca^{2+}) ions also pass from the endoplasmic reticulum to the cytosol. Increasing Ca^{2+} levels in the cytoplasm activate PKC, as well as the phosphorylation of gp91-phox, the subunit of the superoxide-forming NADPH oxidase that binds to Hem (protein-free part of the hemoglobin molecule that binds oxygen). This subunit of NADPH oxidase in the cytosol forms the membrane-binding functional complex in cytoplasmic or phagosomal membranes and generates ROS. The ROS system causes the contents of the granules to be exposed and performs the fusion of nuclear,

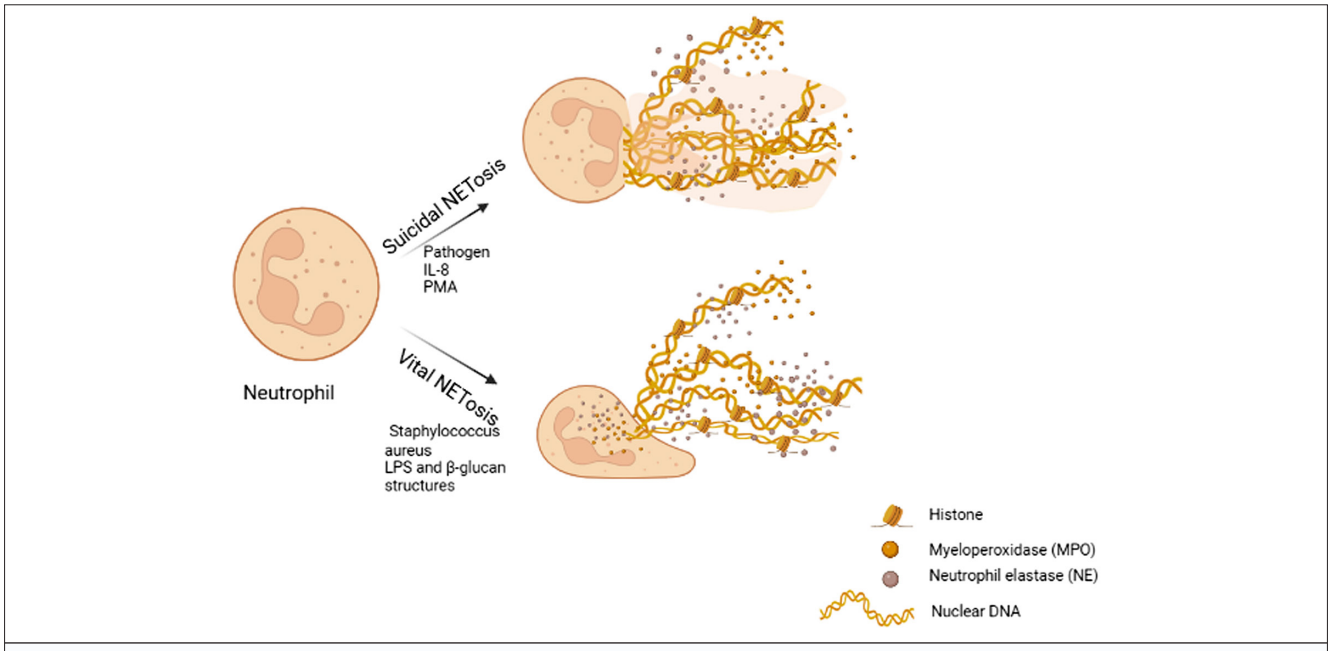

Figure 1. NETosis is of 2 types, suicidal and vital, according to whether the cell integrity of neutrophils is disturbed or not. Suicidal NETosis is the inability of the cell to perform its missions, such as migration and phagocytosis. Since this is a type of cell suicide, cellular kamikaze has also been used to refer to suicidal NETosis. In suicidal NETosis, the nuclear membrane integrity of the cell is impaired, and cytoplasmic chromatin condensation and plasma membrane explosion are also observed. In vital NETosis, no plasma membrane damage or cellular breakdown (lysis) is observed. Neutrophils under vital NETosis begin to become anuclear; nonetheless, plasma membrane integrity is maintained, motility persists, and neutrophils do not lose their ability to phagocytosis. In recent proteomics analyses, neutrophil extracellular traps (NETs) were induced ex vivo by phorbol 12 myristate 13 acetate (PMA), IL-8. After all, according to recent research, lipopolysaccharide (LPS) and β-glucan stimulation have been reported to be important in vital NETosis. Created with BioRender.com.

granular, and cytoplasmic contents. Neutrophil elastase stored in azurophilic granules with MPO is a serine protease that can pass into the nucleus. While NE breaks the histone H1 junction, MPO causes chromatin condensation, revealing extracellular traps. Neutrophil elastase breaks down the actin cytoskeleton, thereby blocking neutrophil phagocytosis. Peptidyl arginine deaminase 4 contributes to chromatin condensation by causing the deamination of histones. The most important event in suicidal NETosis is the inability of the cell to perform its missions, such as migration and phagocytosis due to the impaired plasma integrity, which eventually leads to death. Since this is a type of cell suicide, cellular kamikaze has also been used to refer to suicidal NETosis.[38] In vital NETosis, no plasma membrane damage or cellular breakdown (lysis) is observed; instead, DNA-covered nuclear vesicle bud formation occurs. Neutrophils under vital NETosis begin to become anuclear; nonetheless, plasma membrane integrity is maintained, motility persists, and neutrophils do not lose their ability to phagocytosis.[46] The partial stimulation of vital NETosis has been linked to TLRs and complement factor 3.[47] Unlike suicidal NETosis, the vital NETosis pathway is usually oxidation-independent and faster.[48] After all, according to recent articles, granulocyte macrophage-colony stimulating factor, C5a, and LPS stimulation have been reported to be important in vital NETosis.[49]

Mechanism of Vital NETosis

Unlike conventional (suicidal) NETosis, vital NETosis is a mechanism that arises without destroying the cell integrity of neutrophils. Initially considered to be an alternative pathway of NETosis, vital NETosis was later defined as a different type. In vivo and in vitro studies have shown that in this type of NETosis, which can be stimulated by *Staphylococcus aureus*, the nuclear envelope and vesicular structures take the form of a bubble when examined under an electron microscope. In particular, it has been shown that in vital NETosis, mitochondrial DNA is released into the extracellular field without disturbing the integrity of the cell membrane, just like a catapult. In this form of NETosis, cell surface receptors are stimulated by the recognition of LPS and β-glucan structures and lose cell lobular and multinucleolar shape integrity. At the same time, external and internal nuclear membrane integrity is lost, and the vesicles show a bubble-like appearance. Inside the vesicles are pearl-like sequences of DNA filaments, and dense cytoplasmic granules in the cytoplasm adhere to the plasma membrane. On the cell surface, DNA turns into an extracellular trap, with some cytoplasmic granules fusion with the plasma membrane draining its contents into this extracellular space and combining with DNA. The signaling mechanism to control the formation of NETs has not yet been elucidated. Therefore, there is a need to conduct further studies to evaluate molecular pathways regulating NETosis and explore the importance of neutrophil-mediated biological functions in disease development and health. While NETs can be an effector mechanism of the immune system, they can sometimes alter the immune balance and damage the host. For example, histones fragmented during the formation of NETs have been shown to damage the host. Histones are complementary components of chromatin. Double-stranded DNA is packed into the nucleosome using histones. Extracellular double-stranded DNA formed in the extracellular trap can relate to apoptotic bodies, microparticles, and immune complexes while enveloping the cell like a mesh.[50,51] In addition, NETs are a source of autoantigens,

inducing the production of anti-neutrophilic cytoplasmic antibodies (ANCA).[52] The NET structure has been shown to be responsible for autoimmune and inflammatory diseases, such as acute kidney injury, lupus nephritis, small vessel, and ANCA-associated vasculitis.[53] In many studies based on autoimmune diseases, the roles of NET-associated autoantigens have been demonstrated. For example, α-enolase,[54] annexin A1,[55,56] apolipoprotein A1,[57] C1q,[58] catalase,[59] cathelicidin,[55] citrullinated histones,[58] double-stranded DNA (dsDNA),[60,61] high mobility group box 1 (HMGB1),[60,62,63] cathelicidin LL37,[64] and matrix metallopeptidase 9 (MMP9)[65] have been identified as autoantigens in systemic lupus erythematosus (SLE), and MPO,[66,67] properdin,[68] and tissue factor (TF)[69] in ANCA-associated vasculitis.

NETOSIS IN AUTOIMMUNE AND AUTOINFLAMMATORY DISEASES

Many studies have demonstrated the importance of NET formation in the pathogenesis of autoimmune and autoinflammatory diseases. This review also aims to explain the association between these diseases and NET formation.

Autoimmune Diseases and NETosis

Psoriasis. Psoriasis is a chronic immune-mediated disease characterized by erythematous plaques on the skin. Some patients have psoriatic arthritis accompanied by joint pain and deformations.[70,71] Research has shown that neutrophils that produce pro-inflammatory cytokines, such as IL-6, IL-8, and IL-17, which play roles in psoriatic lesions.[71,72] In keratinocytes, IL-17 increases the expression of LL-37 (mentioned earlier in this paper in terms of its role in the formation of LL-37 NET formation).[73,74] Therefore, though the inflammatory components were formed, NET formation is observed in patients even if there is no infectious agent.[75]

Systemic Lupus Erythematosus. Systemic lupus erythematosus is an autoimmune disease characterized by autoreactive B cells and immune complexes formed due to high levels of interferon (IFN)-α.[76,77] It has been reported that autoantibodies against host nucleic acids are likely to be produced during the formation of NETs in patients with SLE.[40-78] Anti-nuclear antibodies and anti-dsDNA antibodies detected by serological tests are included in the clinical features of the disease. Immune complexes formed by autoantibodies with substance antigens affect the autoimmunity and inflammatory process, determining the severity of the disease.[79,80] The first study demonstrating the effect of NET formation on the pathogenesis of lupus was carried out in the Zychlinsky laboratory in 2010.[81] In that study, the serum samples of a lupus patient and healthy subject were compared, and it was reported that NETs had an immediate gradient in healthy serum, but the stability of this form was maintained in patients with lupus. In addition, a characteristic finding in patients with lupus is that molecules inhibiting the function of DNAase1 also inhibit the degradation of serum NETs.[82] This preservation of NET formation in patients with lupus is considered to be governed by 2 mechanisms: (i) the detection of DNAase1 inhibitors in patient serum and (ii) prevention of the NET gradient through the protection of the DNA backbone with anti-NET antibodies that prevent access to the DNase1 enzyme. In addition, Kim et al[83] have explained that IFN-mediated autoinflammatory diseases, Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature (CANDLE) and STING-Associated Vasculopathy with onset in Infancy, and olacak sanırım Aicardi Goutières syndrome and monogenic forms of SLE (monoSLE) caused by loss-of-function mutations in the DNA nucleases, DNASE1 and DNASE1L3.[83] In addition to this information, it is reported that infiltrated neutrophil and mononuclear cells are seen in the skin in patients with CANDLE.[84] Besides researchers have reported that the prevention of NET degradation has a high risk of NET accumulation in the development of lupus nephritis in SLE.[81] Other studies have shown that neutrophil subgroups vary in a wide range in patients with lupus. Especially in these subgroups, neutrophils prone to NET formation and enzymes that contribute to the formation of NETs, such as MPO and allostasis have been shown to be highly expressed.[85] In addition, in SLE exacerbations, the capacity of patients to clear NETs decreases, and this has been suggested to cause the DNAase inhibition of C1q, and thus the continuity of the disease is maintained by resistant complement activation.[86] In addition, in patients with lupus, macrophages have been shown to be susceptible to the formation of NET-induced NLRP3 inflammasome, which is effective in the course and severity of the disease.[87]

In pediatric cases, B cell origin of autoantibodies has been shown to stimulate plasmacytoid dendritic cells (pDCs) to produce T1-IFN.[86,88] In the light of this information, the activation of active pDCs via TLR9 and the IFN response caused by the expression of pathogenic autoantibodies have been determined by the vascular structure in the components of NET.[85,86,88] The role of NETs in SLE, a disease characterized by inflammation in pediatric cases, is the most researched disease.[89,90]

Rheumatoid Arthritis. Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by resistant synovial inflammation. It particularly involves joints but can also cause complications in solid organs. Although it is known that autoantibodies against citrullinated protein antigens play a role in the pathogenesis of the disease, it is not yet known how the immune system creates these autoantibodies against intracellular targets. The polymorphonuclear cells of patients with RA are more prone to forming NETs and strongly inducing NETosis. It has been reported that the molecules that best explain this situation are citrullinated proteins.[91] Studies evaluating patients with RA have determined that neutrophils show the tendency to form NETs, especially in synovial fluid, and tissue damage caused by innate immune response and adaptive immune response (depending on autoantibodies) can be seen in the joints of these patients.[91,92] Studies showing the presence of MPO, a NET formation protein, in synovial fluid, skin, and rheumatoid nodules in patients with RA also indicate that the formation of NETs is induced in these areas of inflammation.[93-95] It has also been suggested that the formation of NETs and molecules involved in this process may be biomarkers indicating the activity of RA disease, and therefore therapeutic strategies targeting these molecules can be developed.[94-96]

Type 1 Diabetes Mellitus. Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by hyperglycemia caused by damage to pancreatic cells in the islets of Langerhans.[97] One of the main mechanisms that damage pancreatic cells in T1DM is autoantibodies, developing due to the response of immune

cells.[98,99] The development of neutropenia in patients with T1DM poses a great risk, either related to neutrophil sequestration in pancreatic tissue or through neutrophils infiltrating the islets of Langerhans, where the NET formation is then stimulated by TNF-α.[100] In autoimmune diabetes, especially cytokine profiles produced specifically for the inclusion of neutrophils in the field of inflammation have been shown to have an effect on the pathogenesis of the disease and NET formation.[99,100] The pathological roles of the neutrophil however are still unclear in pediatric cases. There is evidence showing a pronounced elevation in circulating protein levels and enzymatic activity of NE and proteinase 3 (PR3) in long-term T1DM pediatric patients. In addition, Klocperk et al[101] evaluated serum levels of MPO, NE, PR3, protein arginine deiminase 4 (PAD4), LL37, and cell-free DNA–histone complexes in long-term pediatric T1D patients. The researchers showed that most biomarkers decreased over time in these pediatric cases but never normalized in long-term patients.

Small Vessel Vasculitis. Anti-neutrophilic cytoplasmic antibodies against MPO and PR3 are important in the diagnosis of small vessel vasculitis, a systemic autoimmune disease. These 2 proteins are components of NET formation, autoantibodies produced against these proteins; increased cell adhesion has a detrimental effect by mediating complement activation and the formation of NETs.[102,103] While high levels of MPO and DNA have been detected in the blood samples of patients with small vessel vasculitis, dense neutrophil infiltrations seen in biopsy samples of patients during active periods of the disease are also important.[104] Anti-neutrophilic cytoplasmic antibody-associated vasculitis is a necrotic vasculitis that has an effect involving small vessels in various organs and systems, such as the kidneys, lungs, skin, and peripheral nervous system. In addition, although anti-neutrophil cytoplasmic antibody-associated vasculitides are rare in children, renal involvement is more common and progression is more severe compared to adults.[105] Anti-neutrophilic cytoplasmic antibodies activate neutrophils, causing anti-microbial and cytotoxic agents to act on the glomerular endothelium, which explains the link between the formation of NETs in humans and ANCA-related vasculitis.[104-106] Therefore, it is considered that the NET components detected in the blood of patients with small vessel vasculitis participate in the circulation in both the B and dendritic cells through TLR signaling pathways. They have been shown to be activated depending on ANCA production.[107,108] The impaired NET formation is likely to support ANCA production and vasculitis development.[99] In patients with active vasculitis, high levels of IFN-α have been reported to be important for pDCs (particularly the interaction of NET-mediated components with pDC TLR9 receptors) activated by NET components.[104] On the other side, ANCA binds to antigens on the surface of TNF-α-induced neutrophils and activates PAD4-dependent NET formation.[109,110] In this process, PAD4 histone converts arginine residuals to citrulline, interrupting the DNA–histone relationship and resulting in the nuclear chromatin condensation required for NET formation. There is a growing number of studies revealing the relationship between vasculitis and NET formation. These studies indicate increased NETs and polymorphonuclear cell death in the blood samples of patients with vasculitis compared to those in remission and healthy controls.[111] Anti-neutrophilic cytoplasmic antibody-associated vasculitides include 3 diseases: (i) Wegener's granulomatosis (granulomatosis with polyangiitis), (ii) microscopic polyangiitis (MPA), and (iii) eosinophilic granulomatosis with polyangiitis (formerly known as Churg-Strauss syndrome).[66] Microscopic polyangiitis is an ANCA-related pathology that affects small vessels, particularly the renal glomeruli.[112] Neutrophil extracellular trap components, especially autoantibodies against MPO, have pioneered the development of MPA.[113] However, it has been reported that immunoglobulins obtained from patients with MPA induce NET release, which is associated with ANCAs with a high affinity for MPO. In addition, patients with MPA have been reported to have low levels of DNAase I in their sera, indicating damage to NET degradation.[109] Neutrophil extracellular trap release is reported to be responsible for renal damage seen in more than 90% of MPA cases.[111] Kraaij et al[114] reported that the uncontrollable formation of NETs occurred independently of the level of ANCAs in the serum samples of patients with ANCA-associated vasculitis. This study was conducted in MPO-ANCA-positive patients; higher levels of NET formation were seen in PR3-ANCA-positive patients. Despite the role of NETs in the pathogenesis of MPA, it has been suggested that NET formation may have different roles in the pathogenesis of granulomatosis with polyangiitis. It has also been reported that histone-associated intraglomerular NETs are the central mechanism of microvascular damage mediating necrosis and effective in the development of necrotizing and crescentic glomerulonephritis.[115]

Antiphospholipid Syndrome. Antiphospholipid syndrome (APS) is usually seen together with SLE, but it is also a primary autoimmune disease. Autoantibodies against phospholipids and phospholipid-binding proteins, such as β2-glycoprotein (β2-GP1), are markers of APS.[116] The risk of thrombosis is particularly high in patients with APS. Therefore, NET formation is considered to play a role in thrombosis.[117] Studies have shown that NET formation in patients with APS occurs with the activation of TLR4 and ROS.[118-120] However, some studies have reported that NETs are not directly effective in the activation of APS; rather, they exhibit their effects indirectly through autoantibodies.[121] For example, the presence of autoantibodies against β2-glycoproteins on the neutrophil surface has been shown in patients with APS.[119] These anti-β2-glycoprotein antibodies have been reported to induce NET formation in patients with APS compared to healthy subjects.[119,120] Another effect of NETs in patients with APS is that they mediate platelet aggregation.[122] Clustered platelets have also been determined to induce the formation of NETs by neutrophils through DAMPs, such as HMGB1.[123]

Multiple Sclerosis. Multiple sclerosis (MS) is a disease involving the central nervous system and is considered to be related to a strong autoimmune response. There are many studies demonstrating the role of NET formation in the pathogenesis of MS.[124] Myeloperoxidase–DNA complexes detected in the serum of patients with MS have been shown not to affect the severity of the disease,[125] while low-density granulocytes (CD14-CD15high) have been detected at similar levels in patients with SLE.[126] However, there are no published studies on whether these low-density granulocytes tend to induce NET formation in patients with MS. In addition to the fact that Naegele et al[127] showed that

there were high levels of IL-8, which induces the formation of NET, in the serums of MS patients, it was also shown that free DNA of NET origin was found in MPO complex form in these patients; NET form may have a role in the pathogenesis of MS. However, the MPO–DNA complex detected in patients with MS plays no role in the activation of the disease since it has been reported that the level of this complex differs between individuals.[127]

Autoinflammatory Diseases and NETosis

Proceeding with immune dysregulation, fever, rash, and, in some cases, mucosal symptoms seen in autoinflammatory diseases are added every day. Autoinflammatory disorders are illnesses defined by systemic inflammations brought on by inflammasomes, independent of infection, as well as autoreactive antibodies or antigen-specific T cell responses.

Gout Disease. This is an autoinflammatory disease that occurs when monosodium urate (MSU) crystals accumulate in the joints. The response of immune system to the accumulation of MSU crystals is to promote leukocyte attack and inflammation to induce NET formation.[128-131] Vedder et al[132] reported that the occurrence of NET in patients with gout disease was not a risk factor for cardiovascular diseases.[132] In addition, Pieterse et al[133] showed ex vivo that MSUs induced NET formation.[133] Vedder et al[132] also suggested that the detection of NETs and MSU crystals in the serum of patients with gout disease may explain the role of NETs in the pathogenesis of the disease.

Inflammatory Bowel Disease. Inflammatory bowel disease (IBD) is a term that describes 2 types of diseases characterized by the uncontrollable inflammation of the gastrointestinal tract: (i) ulcerative colitis and (ii) Crohn's disease. These 2 separate diseases have different etiologies, pathogenesis, and diagnostic criteria. Crohn's disease is characterized by systemic inflammation involving the ileum and colon.[134,135] It has been suggested that ROS generation can truly cause NET formation, although there are only few studies demonstrating the involvement of NET formation in this condition.[73,136,137] In ulcerative colitis, inflammation is limited only to the colon and rectum. In particular, the clustering of NETs in the colon is reported to increase severity.[72] However, it remains unclear how NET formation affects the severity, progression, and incidence of various disorders. With the studies carried out in this direction, it will be possible to provide a better understanding of the real mechanisms of IBD and determine its effects on treatment options.

Familial Mediterranean Fever. Familial Mediterranean Fever (FMF) is an inherited autoinflammatory disease characterized by recurrent fever and serositis caused by mutations in the MEFV (Mediterranean fever) gene encoding the Pyrin protein (TRIM20). Studies have shown psychological and physical stress factors trigger FMF attacks.[138,139] Familial Mediterranean fever is an IL-1β-dependent autoinflammatory disease; therefore, it clinically responds to IL-1β blockade.[140,141] However, neutrophils also play an important role in FMF.[142,143] The pyrin protein, which has a well-known relationship with IL-1β bioreactivity, is expressed at a very high level in neutrophils.[144,145] In addition, Apostolidou et al[146] showed that NETosis formed by neutrophils regulated IL-1β production. In short-term culture studies of neutrophils isolated from patients with FMF, altered gene expressions were observed compared to the neutrophils of healthy subjects, and it was also noted that caspase-1, c-FOS, TLR2, and MMP9 gene expressions increased.[147] According to 1 hypothesis, neutrophil activation is increased in patients with MEFV gene mutations, and it is thought that this condition may be caused by a gene–dose effect of MEFV mutations.[148] There are studies suggesting FMF attacks are induced by stress. In this context, Skendros et al[149] investigated the role of the activation of neutrophils and NET formation in stress-induced inflammatory response in patients with FMF. The authors found that stress-induced proteins, such as REDD1 (regulated in development and DNA damage responses 1), were expressed at high levels in patients with FMF, and neutrophils resulted in autophagy-mediated NET formation due to autophagy resistance, especially in cases in remission. The REDD1 is a protein localized in autolysosomes along with pyrin and NALP3. Due to this mutation observed in pyrin, this association in autolysosomes is disrupted, increasing the secretion of NET-mediated IL-1 β and causing more inflammation.[148]

SUMMARY AND CONCLUSION

In this review, the latest research on the structure, function, and role of NETs in the pathogenesis of autoimmune and autoinflammatory diseases is summarized. We also outlined the NETosis process and its role in the immune response. Specifically, we discussed how the function of NETosis in immune defense later evolved into its role in disease pathogenesis. NETosis is a complex process that is influenced by more than one factor. In many diseases, the excessive NET formation can lead to tissue damage, cause the formation of autoantibodies, and accelerate the progression of the disease. This information will lead to the emergence of new strategies for the prevention and treatment of certain diseases. Future studies should analyze the role of NETosis in autoimmune and autoinflammatory diseases from multiple perspectives. Further research is necessary to confirm what causes the different effects of NETosis in related diseases and to provide a theoretical basis for its treatment.

We know that there are not many studies showing the relationship between NET and autoimmune-autoinflammatory diseases in pediatric cases as much as in adults. In this context, more studies are needed to better understand the role of NETs in the pathophysiology of pediatric autoimmune and autoinflammatory diseases. This will ultimately lead to the development of innovative treatments for pediatric autoimmune and autoinflammatory diseases.

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