

# The Role of Neutrophils Extracellular Traps in Autoimmune Diseases

Seyed Kiarash Aghayan<sup>1,2</sup>, Mohammad Reza Heydari<sup>1</sup>, Javad Hosseini Nejad<sup>3</sup>, Ali Zahiri<sup>4</sup>, Mostafa Eslami Mahmoudabadi<sup>4</sup>, Hadi Esmaeili Ghouvarchinghaleh<sup>2\*</sup>

<sup>1</sup> Department of Veterinary Sciences, Shabestar Branch, Islamic Azad University, Shabestar, Iran

<sup>2</sup> Applied Virology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical sciences, Tehran, Iran

<sup>3</sup> Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>4</sup> Students Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran

\*Corresponding Author: Hadi Esmaeili Ghouvarchinghaleh, Ph.D., Applied Virology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical sciences, Tehran, Iran. Tel: +98-9125075438, Email: h.smali69@yahoo.com

Received February 7, 2024; Accepted March 13, 2024; Online Published March 15, 2024

## Abstract

Some neutrophils are shown to be able to release structures consisting of DNA strands associated with histones, decorated with about 20 proteins. These structures are called Neutrophil Extracellular Traps (NETs). NETosis is the process by which the formation of neutrophil extracellular traps eventually leads to cell death. Indeed, NETosis is a cell death process that is unique from other common types of cell death. Two kinds of NETosis have been identified, vital NETosis and suicidal NETosis. Vital NETosis, unlike suicidal NETosis, occurs a few minutes after neutrophil stimulation. Suicidal NETosis can be dependent on or independent of NADPH oxidase. NADPH-independent NETosis can be induced by calcium ionophores. As long as NETs are set up properly, they play an important role in fighting infections. However, if not properly adjusted, tissue damage and inflammation increase. Furthermore, NETs are involved in some autoimmune diseases.

**Keywords:** Neutrophils, NETosis, Autoimmune, Neutrophil Extracellular Traps, NETs

## 1. Background

Neutrophil Extracellular Traps (NETs) were discovered in 1996, and in 2004, NETosis was first described as an important step in killing bacteria by neutrophils.<sup>1,2</sup> NETs released by active neutrophils are believed to trap and destroy invading microorganisms. Therefore, neutrophils safeguard the host both internally through phagocytosis and externally via NETs. Experiments conducted both in vitro and in vivo demonstrated that gram-negative and gram-positive bacteria, as well as fungi, adhere to DNA fibrils.<sup>3</sup> Over the past few years, it has become more clear that only a fraction of neutrophils can form NETs. This indicates the heterogeneity of neutrophil populations, especially during sterile inflammation.<sup>4</sup> NETosis is the process by which NETs are formed and the formation of these NETs causes cell death. It has also been shown that if there is no balance between the formation of NETs and their destruction, there is a possibility of autoimmune diseases.<sup>5,6</sup> When neutrophils are subjected to NETosis, the nuclear and granular membranes disintegrate, chromatin breaks down and spreads in the cytoplasm and mixes with cytoplasmic proteins. The plasma membrane then ruptures, releasing chromatin adorned with granular proteins into the extracellular space.<sup>7</sup> In the inflammatory environment, NETs expose antigens such as nucleic acids

and proteins that can stimulate the immune response in a sensitive individual.<sup>8</sup> NETosis is characterized by the release of NETs, which are large web-like structures. NETs consist of DNA strands linked with histones and are adorned with approximately 20 different proteins, including Neutrophil Elastase (NE), Myeloperoxidase (MPO), cathepsin G, proteinase 3, high mobility group protein B1, and LL37.<sup>7</sup> Currently, two forms of NETosis have been identified. One is the suicidal NETosis that leads to cell death, and the other is the vital NETosis in which not only the cell survives, but also retains many of its effective functions.<sup>5,7</sup> Both of these processes are believed to perform antimicrobial functions.<sup>3</sup> NETs can play a role in causing chronic inflammatory processes<sup>3</sup> and can also lead to uncontrolled inflammatory responses that lead to tissue damage.<sup>2</sup> Furthermore, NETs are also associated with the resolution of inflammation. Therefore, NETs can be central regulators of inflammation and autoimmunity.<sup>4</sup> NETs are present in various conditions such as infection, malignancy, atherosclerosis, and autoimmune diseases including Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Anti-Neutrophil Cytoplasmic Antibodies (ANCA), Associated Vasculitis (AAV), psoriasis, and gout.<sup>6</sup> The exact molecular mechanisms that cause NETosis are not fully

understood; several pathways have been suggested, such as induction by Reactive Oxygen Species (ROS) generated from NADPH oxidase.<sup>8</sup> Accurate understanding of the various mechanisms and factors that cause these diseases is useful and necessary for their treatment. In this study, the role of neutrophil extracellular traps in causing autoimmune diseases have been discussed.

## 2. Investigation of Neutrophil Extracellular Traps Formation

Neutrophil chromatin undergoes decondensation after stimulation by pathogens. NE and MPO mediate this process.<sup>9</sup> NE and MPO are enzymes stored in azurophilic granules, found in large quantities in NETs. NE causes chromatin to decondensation and MPO enhances this process.<sup>10</sup> Protein Kinase C (PKC) activates NADPH oxidase. Phorbol 12-myristate 13-acetate (PMA) is a NETosis activator that belongs to the PKC family.<sup>9</sup> PMA is a chemical stimulator that is most commonly used to release NETs from neutrophils derived from human blood.<sup>11</sup> ROS induce NETosis. NADPH oxidase is the main source of ROS. Increasing the concentration of  $Ca^{2+}$  in the cytoplasm activates NADPH oxidase. Mitochondrial ROS production also causes this process in some cases. Contrary to previous beliefs that mitochondria do not play an important role in neutrophil function, the role of mitochondrial ROS in NADPH oxidase activation and NETosis induction has been identified.<sup>5</sup> This issue that mitochondria are the source of a significant portion of the nucleic acids in NETs relative to the nucleus has been reported by several studies.<sup>12</sup> Mcl-1 is neutrophils main anti-apoptotic protein that its expression stimulated by PKC. NETosis induction can be associated with apoptosis suppression.<sup>5</sup> The suicidal form of NET formation was initially believed to depend on NADPH oxidase, but it can also occur independently of NADPH oxidase facilitated by calcium influx and mitochondrial ROS production. NADPH oxidase-dependent NET formation is triggered by agonists such as PMA and LPS, with PMA being a non-physiological NADPH oxidase-dependent agonist. Studies have shown that Protein Arginine Deiminase 4 (PAD4) is required for the NADPH oxidase-independent NET formation. It also has no integral part in NADPH-oxidase-dependent NET formation. However, NET formation mediated by LPS and PMA may require PAD4.<sup>13</sup> Inhibition of the PAD4 enzyme disrupts NET formation because it is required for the citrullinating of proteins in neutrophils.<sup>14</sup> NADPH oxidase-independent NET formation can be induced by calcium ionophores, a non-physiological agonists similar to PMA, such as ionomycin.<sup>15</sup> Also, one of the mechanisms that does not require the production of oxidants by cells is the increase of intracellular calcium ionophores.<sup>15</sup> Concanavalin A, like PMA, is a mitogenic stimulant that induce NADPH oxidase-dependent NETosis. These

stimuli also activate the PKC. Prostaglandin E<sub>2</sub> can inhibit NETosis by inhibiting PKC and downstream events that result in NET formation. Factors that modulate NETosis include O<sub>2</sub>, CO<sub>2</sub>, bicarbonate levels, and pH. An acidic environment reduces NETosis.<sup>16</sup> In addition to apoptosis and necrosis, NETosis is also considered a type of cell death for neutrophils. Bacteria are one of the stimulants that can induce NETosis. Although neutrophils release NETs during acute infection with *staphylococcus aureus*, it has been shown that these neutrophils are alive. Neutrophils that survive when NETs are released maintain multitasking capabilities. Rapid release of nuclear DNA is a hallmark of vital NETosis.<sup>11</sup> Vital NETosis can occur in response to LPS-activated platelets. The release of NETs by neutrophils that have been in contact with TLR4-activated platelets has been shown not to damage their membranes, leaving those neutrophils intact.<sup>11,17</sup> Living neutrophils can form NETs following the stimulation of the complement factor 5a receptor. The presence of mitochondrial DNA and the absence of nuclear DNA have been observed in living cells that formed NET.<sup>18</sup> Suicidal NETosis, on the other hand, is a type of cell death process in which NETs are composed of nuclear DNA decorated with various histones, accompanied by rupture of cell membranes and loss of polymorphonuclear neutrophils main functions.<sup>17</sup> While suicidal NETosis requires high doses of PMA and a long time to develop, vital NETosis occurs minutes after neutrophil stimulation with bacteria.<sup>19</sup> As a result, two of the primary differences between vital NETosis and suicidal NETosis can be considered the nature of the stimuli and the duration that takes to release NET.<sup>14</sup> Evidence has shown that NET formation occurs through three mechanisms including NETosis, the release of NET by living cells a) mediated by the vesicular release of nuclear DNA b) formed of mitochondrial DNA.<sup>11</sup> Interestingly, in an infection, ROS formation may play a role through two antimicrobial pathways. The first is the intraphagosomal killing of pathogens in which neutrophils are alive, and the second is killing mediated by NET.<sup>20</sup>

## 3. NETosis and other Forms of Cell Death

NETosis has not been using components of pathways associated with apoptosis or necrosis, and is itself a unique form of cell death.<sup>21</sup> Caspases can be used as an example to show the independence of apoptosis and the formation of NET. Apoptosis is caspase-dependent, while NET formation is independent of apoptotic caspases.<sup>22</sup> The results of a study show that NET formation is independent from apoptosis and necrosis and neither of them is involved in NET formation. It has been shown that the morphological and molecular criteria for apoptosis and necrosis are quite different from NET-induced cell death. One of the differentiation of necrosis

and NET formation is due to nucleus morphological changes between them. The nuclear envelope remains intact in necrosis, but in NETs the nuclear membrane ruptures into a large number of vesicles.<sup>20</sup> Chromatin degradation is essential for NETosis and NET formation. It can be inhibited by inhibition of autophagy or NADPH oxidase. Inhibition of chromatin degradation leads to cell death, which is characterized by apoptotic hallmarks.<sup>23</sup> Comparing pyroptosis with NETosis, it can be noted that pyroptosis is dependent on proinflammatory caspase activity, while NETosis requires neutrophil serine proteases. It should also be noted that the niche of intracellular pathogens is destroyed by pyroptosis, while NETs capture extracellular microorganisms.<sup>24</sup> In addition to pyroptosis, it has been shown that the niche of viruses and bacteria inside the cell is also destroyed by the apoptosis and necroptosis.<sup>25</sup> Decomposition of chromatin, disintegration of the nuclear membrane, and rupture of the plasma membrane to release NETs are events that occur during NETosis (suicidal NETosis)-mediated cell death, unlike apoptosis and necrosis.<sup>26</sup> It is also stated that the granular membrane disintegrates during NETosis, but the plasma does not lose its integrity.<sup>27</sup> The results of a study examining the different pathways of NETs formation caused by the five stimuli including PMA, calcium ionophore A23187, bacterial toxin nigericin, dimorphic fungus *Candida albicans* and, gram-positive bacteria Group B *Streptococcus* show that all five pathways have similar properties. In addition, as mentioned earlier, NETosis has been shown to be a unique form of cell death, different from apoptosis and necrosis.<sup>21</sup> Another type of cell death induced by lipid peroxidation is called ferroptosis. The components of the ferroptosis machinery are thought to have a role in the formation of NET. The results of a study showed the acceleration of NET formation by a ferroptosis inducer but it was concluded that this substance is independent of cystine transporter component glutamate/cystine xCT

antiporter.<sup>22</sup> Research in recent years has shown that NETosis induction requires autophagy, and NET formation is closely related to autophagy.<sup>26,27</sup>

#### 4. Relationship between Neutrophil Extracellular Traps and Autoimmune Diseases

NETs play a valuable role in fighting infections, but when the correct regulation of these NETs in the host is disturbed, direct tissue damage and inflammation increase.<sup>28</sup> Factors that cause NET formation include IL-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , IL-8, IFN- $\alpha$ , monosodium urate (MSU) crystals, and alum.<sup>29</sup> It has been hypothesized that NETs are considered as strong inducers of autoimmunity<sup>30</sup> (Table 1). In these inflammatory diseases, the immune system loses its tolerance to autoantigens resulting in effector mechanisms.<sup>31</sup> There is ample evidence for the role of NETosis in various lung diseases, cancer, and autoimmune diseases. NETs have also been shown to have the ability to perpetuate autoimmunity because they contain autoantigens.<sup>30</sup> Neutrophils by engulfing dead cells and cell debris help eliminate a source of sterile inflammation. Therefore, it can be stated that although neutrophils have proinflammatory function, they also help to eliminate inflammation.<sup>19</sup> Neutrophils modulate inflammation and efficiently clear microorganisms during infection by two forms of cell death called apoptosis and NETosis.<sup>32</sup> NET formation also occurs in various forms of vasculitis, and the production of ANCAs against MPO and PR3, which are components of NET, occurs under conditions of vasculitis and autoimmunity.<sup>33</sup> Some forms of cell death, such as apoptosis, necrosis, and NETs, are associated with autoimmunity. One of the enzymes that plays an essential role in the opsonization of NETs for clearance by macrophages is DNase I. This enzyme is also important for the degradation of NETs and C1q.<sup>34</sup> In preventing NET pathology during chronic inflammation, DNase is effective.<sup>35</sup>

**Table 1.** Aspects of Neutrophil Extracellular Traps (NETs) in different autoimmune diseases

| Aspect             | Rheumatoid Arthritis (RA)  | Systemic Lupus Erythematosus (SLE)   | Psoriasis  | Gout   |
|--------------------|--|--|--|--|
| NET Inducers       | RF, ACPAs, antibodies/molecules stimulating the immune system        | Increased mitochondrial superoxide production, Production increased in low-density granulocytes (LDGs) | pDC-derived IFN-I, PMA, TNF- $\alpha$ , <i>S. aureus</i>                   | MSU crystals and requires autophagy, PI3K signaling, endosomal acidification |
| Role in Disease    | Contributes to joint damage, formation of citrullinated autoantigens | Causes systemic inflammation, protects against DNase1  | Increases NET formation in inflamed skin, correlates with disease severity | MSU crystals form aggNETs that have been suggested to limit inflammation     |
| Cytokines Involved | TNF- $\alpha$  | IFN-I, IFN- $\gamma$ , IL-6, IL-8, TNF- $\alpha$   | IL-36, TLR4  | IL-1 $\beta$   |
| Treatment          | Targeting NETs or neutrophil elastase may slow cartilage damage      | DNase1 to degrade NETs   | SLPI treatment to control NET formation                                    | Suramin, PPADS, MRS2578 to inhibit P2Y receptor                              |

#### 4.1. Rheumatoid Arthritis

Antibodies or molecules that stimulate the immune system stimulate the formation of NET in the peripheral blood of patients with RA. Rheumatoid Factor (RF) and

Anti-Citrullinated Protein Antibodies (ACPAs) also support the formation of NET in RA.<sup>36</sup> It seems that ACPA formation is a pathogenic event in the progression of RA.<sup>37</sup> ACPA is a diagnostic feature of RA.<sup>38</sup> Since

ACPA can detect citrullinated proteins and NETosis is a process associated with citrullination, NET may play a key role in the pathogenesis of RA.<sup>39</sup> Aggrecan is an example of a potent citrullinated autoantigen in RA, a protein in the extracellular matrix of articular cartilage.<sup>40</sup> Cathepsins, MPO, MMP8, MMP9, LCN2, and PADI2 are NET proteins with high levels in RA synovial fluid.<sup>41</sup> It is possible that the proteins such as myeloperoxidase, neutrophil elastase, and cathepsin G that cause joint damage, and are present in the synovial fluid due to excessive NETosis.<sup>38</sup> High levels of peptidyl arginine deiminases are expressed by neutrophils, and NETosis is associated with active peptidyl arginine deiminases release. The production of citrullinated autoantigens, following protein deimination by peptidyl arginine deiminases, is a crucial step in the autoimmune response in ACPA-positive RA patients.<sup>39,42</sup> It has been shown that NET formation is much higher in RF (+) than in RF (-) RA patients. It has also been shown that control polymorphonuclear cells isolated from healthy donors tend to form fewer NETs spontaneously compared to RA polymorphonuclear cells.<sup>43</sup> MPO-DNA complexes (remnants of NET) have been shown to be higher in patients with RA than in healthy individuals.<sup>37</sup> MPO-DNA levels are associated with increased neutrophil counts, positive RF, and positive anti-citrullinated protein/peptide antibodies in RA patients.<sup>41</sup> The results of a study show that the progression of cartilage damage can be slowed down by treatments that target NETs or neutrophil elastase. These treatments can also reduce the inability of RA.<sup>40</sup> The results of another study show that anti-NET antibodies (ANETA) are often present in patients with RA. ANETA was much higher in RF (+) patients/<sup>44</sup> MPO and PR3 in ANCA-associated vasculitis, dsDNA in SLE, and autoantibodies to citrullinated protein antigens in RA are autoantigens that may be sourced from proteins found in NETs. Interestingly, it is stated that the levels of inflammatory cytokines, such as TNF- $\alpha$  and IL-17, and pathogenic autoantibodies to citrullinated protein antigen, are increased years before clinical diagnosis in patients with RA.<sup>8</sup>

#### **4.2. Systemic Lupus Erythematosus**

Another autoimmune disease that patients with it may have inflammation in the joints, kidneys, skin, and brain is Systemic Lupus Erythematosus (SLE). In this disease, healthy tissues of the body are attacked by the immune system.<sup>45</sup> Improper activation of the immune system in this disease, which causes systemic inflammation, is due to the presence of autoantigens. In lupus patients' kidneys, NETs have been found. Production of mitochondrial superoxide is crucial for NET formation. Production of this substance in lupus Low-Density Granulocytes (LDGs) is increased compared to normal density granulocytes in both lupus disease and healthy patients.<sup>46</sup>

Chromatin and lactoferrin, myeloperoxidase, proteinase 3, and elastase, which are neutrophil proteins, are targeted by antibodies produced in SLE patients. As mentioned earlier, the degradation of NETs required serum DNase1. A specific DNase1 inhibitor has been observed in the serum of some SLE patients. High titers of NETs-binding antibodies that protect them against DNase1 have also been observed in the serum of other SLE patients.<sup>47</sup> According to a study by Campbell et al. on a murine model, it has been shown that, contrary to the idea that the pathogenesis of SLE is driven by NET, a source of autoantigen, SLE can proceed without the formation of NET and the dependence of NET formation on Nox2 is not a major cause of disease pathology.<sup>45</sup> LDGs have a greater capacity to synthesize proinflammatory cytokines. IFN-I is one of these cytokines and its increased activity is associated with the endothelial dysfunction development in SLE.<sup>48,49</sup> IFN  $\gamma$ , IL-6, IL-8, and TNF- $\alpha$  are other proinflammatory cytokines produced by LDGs that are important in the pathogenesis of SLE.<sup>50</sup> The small vesicles that form as a result of cell membrane germination during stimulation or in the late stages of apoptosis are called microparticles. Dieker et al. showed that unlike plasma microparticles isolated from healthy individuals or RA patients, SLE plasma microparticles can activate Plasmacytoid Dendritic Cells (PDCs) derived from the blood. Dieker et al. have also shown that the NET formation can be increased in SLE patients with high levels of circulating apoptotic microparticles.<sup>51</sup> Decreased DNA destruction ability has been observed in SLE patients. In addition, it has been confirmed that NETs destroy ability is not present in the serum of a subgroup of SLE patients.<sup>52</sup> Apoptosis is more common in SLE neutrophils.<sup>50</sup> Neutrophil accumulation and neutrophil death, including apoptosis, necrosis, and NETosis, have been shown to increase in mice with milk fat globule EGF factor 8 (MFG-E8) deficiency that are exposed to pristane. The absence of MFG-E8 leads to NET formation, autoantibody synthesis, and glomerulonephritis.<sup>53</sup> It has been observed that in SLE patients compared to controls, ubiquitination in NETs is reduced. Protein degradation, for example, is a post-translational modification. These modifications are called ubiquitination, which in addition to protein degradation can affect various cellular processes by modifying protein function and gene transcription.<sup>54</sup> It has been indicated that the levels of basal autophagy in peripheral blood neutrophils were lower in healthy individuals and inactive SLE patients compared with patients with SLE.<sup>55</sup>

#### **4.3. Psoriasis**

Psoriasis is a chronic disease that mostly affects the skin and joints and affects many people.<sup>56</sup> One of the important factors involved in psoriasis is pDC-derived IFN-I. It has been shown that Secretory Leukocyte

Proteinase Inhibitor (SLPI) associated with NETs are more present in the sera-treated neutrophils of psoriasis patients than in PMA-stimulated cells.<sup>57</sup> In psoriasis skin lesions, SLPI might bind to DNA and neutrophil elastase and produce IFN-I after activation of pDCs.<sup>58</sup> Stimulation of human neutrophils by PMA, TNF- $\alpha$ , and *staphylococcus aureus* can cause NET formation in them, a process that is greatly reduced by SLPI treatment. Findings from studies highlight the controlling role of SLPI in NET formation.<sup>59</sup> Although an increase in NET formation induction ability has been observed in control neutrophils by psoriasis sera, their ability to degrade the NET remains normal. It has been indicated that the severity of psoriasis in peripheral blood is correlated with the amount of NET.<sup>60</sup> CD11b and CD66b, which have been shown to increase in active neutrophils, are less in normal healthy controls as well as in patients with moderate psoriasis than in patients with severe psoriasis. Higher levels of LL-37, a component of NETs that is antimicrobial, were observed in keratinocytes of psoriasis lesions.<sup>61</sup> The activation of IL-36 and toll-like receptor 4 (TLR4) signaling by neutrophils through the release of NETs has been shown to enhance skin inflammation. The amount of neutrophils and NETs in inflamed skin were reduced in IMQ mice treated with a neutralizing LCN2 antibody. In psoriasis, the gene LCN2 is prominently expressed.<sup>62</sup>

### 5. The Relationship between Gout and NETosis

The results of a new study show the presence of NETs in the peripheral blood of gout patients.<sup>63</sup> Gout is an acute inflammatory disease characterized by the deposition of extracellular pathogens called MSU crystals. These crystals can trigger immunological responses and can also induce the formation of NET, one of the strategies to counteract extracellular agents.<sup>64</sup> It has been shown that the release of NETs in neutrophil granulocytes mediated by MSU crystals requires autophagy, PI3K signaling, and endosomal acidification.<sup>65</sup> One of the cells that play an important role in the removal of these NETs is CD14<sup>+</sup> macrophages in the synovial fluid.<sup>64</sup> Activation of NLRP3 inflammasome occurs due to the uptake of MSU crystals, one of the strongest inducers of NETosis, by macrophages.<sup>64,66,67</sup> It should be noted that not all patients with hyperuricemia have gout, but serum urate is directly associated with gout. Neutrophils treated with MSU crystals have been shown to cause NET formation as well as synovial fluid and peripheral neutrophils in patients with acute gouty arthritis.<sup>68</sup> IL-1 $\beta$  is a proinflammatory cytokine that regulates neutrophil functions, can be secreted by macrophages due to the uptake of MSU crystals<sup>66,68</sup> and this cytokine has been reported to increase NET release. MSU crystals form aggregated NETs (aggNETs) that have been suggested to limit inflammation.<sup>65</sup> The release of ATP and lactoferrin from activated neutrophils increases this formation<sup>69</sup> and

reports indicated that this formation depends on the ROS production.<sup>70</sup> The microcrystals stimulate neutrophils to release NETs, which at low densities neutrophils form NETs, and at high densities neutrophils form aggNETs.<sup>70</sup> The formation of aggNET stops inflammation by destroying proinflammatory cytokines and densely packed crystals. Studies have shown that the prerequisite for NET formation is phagocytosis of MSU crystals by neutrophil granulocytes.<sup>65</sup> It has been shown that NETosis caused by MSU crystals can be partially inhibited by blocking ROS with various antioxidants.<sup>67</sup> Data from a study show that MSU crystals in all three granulocyte lineages (NETs, EETs, and BETs) can induce the formation of extracellular DNA traps, but they do not have the ability to induce extracellular DNA traps in monocytes. It has also been shown that DNA externalization does not require the adsorption of these crystals.<sup>71</sup> P2Y and P2Y6 are purinergic receptors that studies described the effect of these receptors on the formation of NETs induced by MSU crystals and they have been suggested to be involved in the formation of NETs.<sup>70</sup> Suramin, PPADS and MRS2578 inhibit the P2Y receptor. These substances can limit the formation of NET induced by MSU.<sup>6</sup>

### 6. Conclusion

It is now known that mitochondria are involved in inducing NETosis. Mitochondrial ROS activates NADPH oxidase. Two forms have been identified for NETosis. Vital NETosis and Suicidal NETosis. Suicidal NETosis has two forms. The first is dependent on NADPH oxidase and the second is independent on NADPH oxidase. PMA and LPS are NET-forming agonists that are dependent on NADPH oxidase. Calcium ionophores can also cause NET formation independent of NADPH oxidase. NETosis has been identified as a unique form of cell death and NET formation is independent from apoptosis and necrosis. DNase I is an important enzyme for the degradation of NETs. A specific inhibitor of this enzyme has been observed in the serum of some SLE patients. NETs are found in the kidneys of lupus patients. Decreased DNA degradability has also been observed in SLE. NET proteins such as Cathepsins, MPO, MMP9, etc., have been observed in large amounts in the synovial fluid of RA patients. It has also been observed that MPO-DNA complexes are higher in RA patients than in healthy individuals. Evidence suggests that NET formation may be associated with RA. Psoriasis is another autoimmune disease that has been observed to increase the induction of the ability to form NET in control neutrophils by the psoriasis sera. But it should be noted that their ability to destroy the NET is normal. The severity of psoriasis in peripheral blood has also been shown to be associated with NET levels. MSU crystals can form NET. Neutrophils treated with MSU crystals confirm this fact.

An important point is that serum urate is directly related to gout. Evidence from studies shows that NETs can be involved in the development of various diseases, including autoimmune diseases. In this review, the relationship between four autoimmune diseases and NET formation was investigated. Evidence from studies suggests that NETs may play an important role in causing these autoimmune diseases.

#### Author Contributions

The resource and writing-original draft preparation were carried out by HEGG, SKA, and MRH. The writing review and editing were performed by HEGG, SKA, SJHN, and AZ. The supervision was done by HEGG. The whole manuscript was read and approved by all authors.

#### Conflict of Interest Disclosures

All authors declared that they have no conflict of interest.

#### References

- He Y, Yang FY, Sun EW. Neutrophil extracellular traps in autoimmune diseases. *Chin Med J*. 2018;131(13):1513-9. doi:10.4103/0366-6999.235122
- Mutua V, Gershwin LJ. A review of neutrophil extracellular traps (NETs) in disease: potential anti-NETs therapeutics. *Clin Rev Allergy Immunol*. 2021;61(2):194-211. doi:10.1007/s12016-020-08804-7
- Pinegin B, Vorobjeva N, Pinegin V. Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity. *Autoimmun Rev*. 2015;14(7):633-40. doi:10.1016/j.autrev.2015.03.002
- Fousert E, Toes R, Desai J. Neutrophil extracellular traps (NETs) take the central stage in driving autoimmune responses. *Cells*. 2020;9(4):915. doi:10.3390/cells9040915
- Vorobjeva N, Cheryak B. NETosis: Molecular mechanisms, role in physiology and pathology. *Biochemistry*. 2020;85(10):1178-1190. doi:10.1134/S0006297920100065
- Lee KH, Kronbichler A, Park DD, Park Y, Moon H, Kim H, et al. Neutrophil extracellular traps (NETs) in autoimmune diseases: a comprehensive review. *Autoimmun Rev*. 2017;16(11):1160-73. doi:10.1016/j.autrev.2017.09.012
- Yang H, Biermann MH, Brauner JM, Liu Y, Zhao Y, Herrmann M. New insights into neutrophil extracellular traps: mechanisms of formation and role in inflammation. *Front Immunol*. 2016;7:302. doi:10.3389/fimmu.2016.00302
- Barnado A, Crofford LJ, Oates JC. At the Bedside: Neutrophil extracellular traps (NETs) as targets for biomarkers and therapies in autoimmune diseases. *J Leukoc Biol*. 2016;99(2):265-78. doi:10.1189/jlb.5BT0615-234R
- Kumar S, Gupta E, Kaushik S, Jyoti A. Neutrophil extracellular traps: formation and involvement in disease progression. *Iran J Allergy Asthma Immunol*. 2018;208-20.
- Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol*. 2010;191(3):677-91. doi:10.1083/jcb.201006052
- de Buhr N, von Kuckritz-Blickwede M. How neutrophil extracellular traps become visible. *J Immunol Res*. 2016;2016(1):4604713. doi:10.1155/2016/4604713
- Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ*. 2018;25(3):486-541. doi:10.1038/s41418-017-0012-4
- Ravindran M, Khan MA, Palaniyar N. Neutrophil extracellular trap formation: physiology, pathology, and pharmacology. *Biomolecules*. 2019;9(8):365. doi:10.3390/biom9080365
- Yipp BG, Kubes P. NETosis: how vital is it?. *Blood*. 2013;122(16):2784-94. doi:10.1182/blood-2013-04-457671
- Parker H, Dragunow M, Hampton MB, Kettle AJ, Winterbourn CC. Requirements for NADPH oxidase and myeloperoxidase in neutrophil extracellular trap formation differ depending on the stimulus. *J Leukoc Biol*. 2012;92(4):841-9. doi:10.1189/jlb.1211601
- Sollberger G, Tilley DO, Zychlinsky A. Neutrophil extracellular traps: the biology of chromatin externalization. *Dev Cell*. 2018;44(5):542-53. doi:10.1016/j.devcel.2018.01.019
- Zhou E, Silva LM, Conejeros I, Velásquez ZD, Hirz M, Gärtner U, et al. Besnoitia besnoiti bradyzoite stages induce suicidal-and rapid vital-NETosis. *Parasitology*. 2020;147(4):401-9. doi:10.1017/S0031182019001707
- Yousefi S, Mihalache C, Kozłowski E, Schmid I, Simon H-U. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ*. 2009;16(11):1438-44. doi:10.1038/cdd.2009.96
- Jorch SK, Kubes P. An emerging role for neutrophil extracellular traps in noninfectious disease. *Nat Med*. 2017;23(3):279-87. doi:10.1038/nm.4294
- Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007;176(2):231-41. doi:10.1083/jcb.200606027
- Kenny EF, Herzig A, Krüger R, Muth A, Mondal S, Thompson PR, et al. Diverse stimuli engage different neutrophil extracellular trap pathways. *Elife*. 2017;6:e24437. doi:10.7554/eLife.24437
- Rosazza T, Warner J, Sollberger G. NET formation—mechanisms and how they relate to other cell death pathways. *FEBS J*. 2021;288(11):3334-50. doi:10.1111/febs.15589
- Remijsen Q, Berghe TV, Wirawan E, Asselbergh B, Parthoens E, De Rycke R, et al. Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. *Cell Res*. 2011;21(2):290-304. doi:10.1038/cr.2010.150
- Sollberger G, Choidas A, Burn GL, Habenberger P, Di Lucrezia R, Kordes S, et al. Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci Immunol*. 2018;3(26):eaar6689. doi:10.1126/sciimmunol.aar6689
- Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. *Nat Rev Immunol*. 2017;17(3):151. doi:10.1038/nri.2016.147
- Skendros P, Mitroulis I, Ritis K. Autophagy in neutrophils: from granulopoiesis to neutrophil extracellular traps. *Front Cell Dev Biol*. 2018;6:109. doi:10.3389/fcell.2018.00109
- Remijsen Q, Kuijpers T, Wirawan E, Lippens S, Vandenabeele P, Berghe TV. Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. *Cell Death Differ*. 2011;18(4):581-8. doi:10.1038/cdd.2011.1
- Skopelja-Gardner S, Jones JD, Rigby WF. "NETtling" the host: Breaking of tolerance in chronic inflammation and chronic infection. *J Autoimmun*. 2018;88:1-10. doi:10.1016/j.jaut.2017.10.008
- Dwivedi N, Radic M. Burning controversies in NETs

- and autoimmunity: the mysteries of cell death and autoimmune disease. *Autoimmunity*. 2018;51(6):267-80. doi:10.1080/08916934.2018.1523395
30. Granger V, Peyneau M, Chollet-Martin S, De Chaisemartin L. Neutrophil extracellular traps in autoimmunity and allergy: immune complexes at work. *Front Immunol*. 2019;10:2824. doi:10.3389/fimmu.2019.02824
  31. Castanheira FV, Kubes P. Neutrophils and NETs in modulating acute and chronic inflammation. *Blood*. 2019;133(20):2178-85. doi:10.1182/blood-2018-11-844530
  32. Andrade F, Darrah E. NETs: the missing link between cell death and systemic autoimmune diseases? *Front Immunol*. 2013;3:428. doi:10.3389/fimmu.2012.00428
  33. Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*. 2013;13(3):159-75. doi:10.1038/nri3399
  34. Mistry P, Kaplan MJ. Cell death in the pathogenesis of systemic lupus erythematosus and lupus nephritis. *Clin Immunol*. 2017;185:59-73. doi:10.1016/j.clim.2016.08.010
  35. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol*. 2018;18(2):134. doi:10.1038/nri.2017.105
  36. Song W, Ye J, Pan N, Tan C, Herrmann M. Neutrophil extracellular traps tied to rheumatoid arthritis: points to ponder. *Front Immunol*. 2021;11:578129. doi:10.3389/fimmu.2020.578129
  37. Wang W, Peng W, Ning X. Increased levels of neutrophil extracellular trap remnants in the serum of patients with rheumatoid arthritis. *Int J Rheum Dis*. 2018;21(2):415-21. doi:10.1111/1756-185X.13226
  38. Apel F, Zychlinsky A, Kenny EF. The role of neutrophil extracellular traps in rheumatic diseases. *Nat Rev Rheumatol*. 2018;14(8):467-75. doi:10.1038/s41584-018-0039-z
  39. Ribon M, Seninet S, Mussard J, Sebbag M, Clavel C, Serre G, et al. Neutrophil extracellular traps exert both pro-and anti-inflammatory actions in rheumatoid arthritis that are modulated by C1q and LL-37. *J Autoimmun*. 2019;98:122-31. doi:10.1016/j.jaut.2019.01.003
  40. Carmona-Rivera C, Carlucci PM, Goel RR, James E, Brooks SR, Rims C, et al. Neutrophil extracellular traps mediate articular cartilage damage and enhance cartilage component immunogenicity in rheumatoid arthritis. *JCI Insight*. 2020;5(13):e139388. doi:10.1172/jci.insight.139388
  41. Chapman EA, Lyon M, Simpson D, Mason D, Beynon RJ, Moots RJ, et al. Caught in a trap? Proteomic analysis of neutrophil extracellular traps in rheumatoid arthritis and systemic lupus erythematosus. *Front Immunol*. 2019;10:423. doi:10.3389/fimmu.2019.0423
  42. Spengler J, Lugonja B, Jimmy Ytterberg A, Zubarev RA, Creese AJ, Pearson MJ, et al. Release of active peptidyl arginine deiminases by neutrophils can explain production of extracellular citrullinated autoantigens in rheumatoid arthritis synovial fluid. *Arthritis Rheumatol*. 2015;67(12):3135-45. doi:10.1002/art.39313
  43. Papadaki G, Kambas K, Choulaki C, Vlachou K, Drakos E, Bertsias G, et al. Neutrophil extracellular traps exacerbate Th1-mediated autoimmune responses in rheumatoid arthritis by promoting DC maturation. *Eur J Immunol*. 2016;46(11):2542-54. doi:10.1002/eji.201646542
  44. de Bont CM, Stokman ME, Faas P, Thurlings RM, Boelens WC, Wright HL, et al. Autoantibodies to neutrophil extracellular traps represent a potential serological biomarker in rheumatoid arthritis. *J Autoimmun*. 2020;113:102484. doi:10.1016/j.jaut.2020.102484
  45. Campbell AM, Kashgarian M, Shlomchik MJ. NADPH oxidase inhibits the pathogenesis of systemic lupus erythematosus. *Sci Transl Med*. 2012;4(157):157ra141. doi:10.1126/scitranslmed.3004801
  46. Salemm R, Peralta LN, Meka SH, Pushpanathan N, Alexander JJ. The role of NETosis in systemic lupus erythematosus. *J Cell Immunol*. 2019;1(2):33. doi:10.33696/immunology.1.008
  47. Hakkim A, Fürnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A*. 2010;107(21):9813-8. doi:10.1073/pnas.0909927107
  48. Knight JS, Subramanian V, O'Dell AA, Yalavarthi S, Zhao W, Smith CK, et al. Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. *Ann Rheum Dis*. 2015;74(12):2199-206. doi:10.1136/annrheumdis-2014-205365
  49. Smith CK, Kaplan MJ. The role of neutrophils in the pathogenesis of systemic lupus erythematosus. *Curr Opin Rheumatol*. 2015;27(5):448-53. doi:10.1097/BOR.0000000000000197
  50. Wirestam L, Arve S, Linge P, Bengtsson AA. Neutrophils—Important Communicators in Systemic Lupus Erythematosus and Antiphospholipid Syndrome. *Front Immunol*. 2019;10:2734. doi:10.3389/fimmu.2019.02734
  51. Dieker J, Tel J, Pieterse E, Thielen A, Rother N, Bakker M, et al. Circulating apoptotic microparticles in systemic lupus erythematosus patients drive the activation of dendritic cell subsets and prime neutrophils for NETosis. *Arthritis Rheumatol*. 2016;68(2):462-72. doi:10.1002/art.39417
  52. Leffler J, Ciacma K, Gullstrand B, Bengtsson AA, Martin M, Blom AM. A subset of patients with systemic lupus erythematosus fails to degrade DNA from multiple clinically relevant sources. *Arthritis Res Ther*. 2015;17(1):1-10. doi:10.1186/s13075-015-0726-y
  53. Huang W, Wu J, Yang H, Xiong Y, Jiang R, Cui T, et al. Milk fat globule-EGF factor 8 suppresses the aberrant immune response of systemic lupus erythematosus-derived neutrophils and associated tissue damage. *Cell Death Differ*. 2017;24(2):263-75. doi:10.1038/cdd.2016.115
  54. Barrera-Vargas A, Gymež-Martín D, Carmona-Rivera C, Merayo-Chalico J, Torres-Ruiz J, Manna Z, Hasni S, Alcocer-Varela J, Kaplan MJ. Differential ubiquitination in NETs regulates macrophage responses in systemic lupus erythematosus. *Ann Rheum Dis*. 2018;77(6):944-50. doi:10.1136/annrheumdis-2017-212617
  55. Frangou E, Vassilopoulos D, Boletis J, Boumpas DT. An emerging role of neutrophils and NETosis in chronic inflammation and fibrosis in systemic lupus erythematosus (SLE) and ANCA-associated vasculitides (AAV): Implications for the pathogenesis and treatment. *Autoimmun Rev*. 2019;18(8):751-60. doi:10.1016/j.autrev.2019.06.011
  56. Zhang B, Lai RC, Sim WK, Choo ABH, Lane EB, Lim SK. Topical Application of Mesenchymal Stem Cell Exosomes Alleviates the Imiquimod Induced Psoriasis-Like Inflammation. *Int J Mol Sci*. 2021;22(2):720. doi:10.3390/ijms22020720
  57. Skrzeczynska-Moncznik J, Włodarczyk A, Zabiegło K, Kapinska-Mrowiecka M, Marewicz E, Dubin A, et al. Secretory leukocyte proteinase inhibitor-competent DNA deposits are potent stimulators of plasmacytoid dendritic cells: implication for psoriasis. *J Immunol*. 2012;189(4):1611-7. doi:10.4049/jimmunol.1103293
  58. Chiang C-C, Cheng W-J, Korinek M, Lin C-Y, Hwang

- T-L. Neutrophils in psoriasis. *Front Immunol.* 2019;10:2376. doi:10.3389/fimmu.2019.02376
59. Zabieglo K, Majewski P, Majchrzak-Gorecka M, Wlodarczyk A, Grygier B, Zegar A et al. The inhibitory effect of secretory leukocyte protease inhibitor (SLPI) on formation of neutrophil extracellular traps. *J Leukoc Biol.* 2015;98(1):99-106. doi:10.1189/jlb.4AB1114-543R
60. Hu SC-S, Yu H-S, Yen F-L, Lin C-L, Chen G-S, Lan C-CE. Neutrophil extracellular trap formation is increased in psoriasis and induces human  $\beta$ -defensin-2 production in epidermal keratinocytes. *Sci Rep.* 2016;6(1):1-14. doi:10.1038/srep29505
61. Wang WM, Jin HZ. Role of neutrophils in psoriasis. *J Immunol Res.* 2020;2020(1):3709749. doi:10.1155/2020/3709749
62. Shao S, Fang H, Dang E, Xue K, Zhang J, Li B, et al. Neutrophil extracellular traps promote inflammatory responses in psoriasis via activating epidermal TLR4/IL-36R crosstalk. *Front Immunol.* 2019;10:746. doi:10.3389/fimmu.2019.00746
63. Vedder D, Gerritsen M, Duvvuri B, van Vollenhoven R, Nurmohamed M, Lood C. Neutrophil activation identifies patients with active polyarticular gout. *Arthritis Res Ther.* 2020;22(1):148. doi:10.1186/s13075-020-02244-6
64. Jeong JH, Choi SJ, Ahn SM, Oh JS, Kim YG, Lee CK, et al. Neutrophil extracellular trap clearance by synovial macrophages in gout. *Arthritis Res Ther.* 2021;23:88. doi:10.1186/s13075-021-02472-4
65. Rada B. Neutrophil extracellular traps and microcrystals. *J Immunol Res.* 2017;2017(1):2896380. doi:10.1155/2017/2896380
66. Schauer C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, et al. Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. *Nat Med.* 2014;20(5):511-7. doi:10.1038/nm.3547
67. Schorn C, Janko C, Krenn V, Zhao Y, Munoz LE, Schett G, et al. Bonding the foe—NETting neutrophils immobilize the pro-inflammatory monosodium urate crystals. *Front Immunol.* 2012;3:376. doi:10.3389/fimmu.2012.00376
68. Mitroulis I, Kambas K, Ritis K. Neutrophils, IL-1 $\beta$ , and gout: is there a link?. *Semin Immunopathol.* 2013;35:501-12. doi:10.1007/s00281-013-0361-0
69. Maueröder C, Kienhöfer D, Hahn J, Schauer C, Manger B, Schett G, et al. How neutrophil extracellular traps orchestrate the local immune response in gout. *J Mol Med.* 2015;93:727-34. doi:10.1007/s00109-015-1295-x
70. Li Y, Cao X, Liu Y, Zhao Y, Herrmann M. Neutrophil extracellular traps formation and aggregation orchestrate induction and resolution of sterile crystal-mediated inflammation. *Front Immunol.* 2018;9:1559. doi:10.3389/fimmu.2018.01559
71. Schorn C, Janko C, Latzko M, Chaurio R, Schett G, Herrmann M. Monosodium urate crystals induce extracellular DNA traps in neutrophils, eosinophils, and basophils but not in mononuclear cells. *Front Immunol.* 2012;3:277. doi:10.3389/fimmu.2012.00277