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Review Article

Neutrophil Extracellular Traps in Inflammatory and Autoimmune Diseases and Cancer

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ABSTRACT

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Neutrophils are innate immune system phagocytes that play a central role in immunity defense. They are equipped with effective antimicrobial that is mainly stored in specialized granules. Considering that, it can also damage host tissue. Neutrophil deployment is heavily regulated through various strategies, including phagocytosis, reactive oxygen species, production degranulation, and the formation of neutrophil extracellular traps (NET). This review article will discuss its role in inflammatory, autoimmune diseases, and cancer and place it as a therapeutic target. It depicts that NET formation includes a suicide program morphologically different from other types of cell death, such as apoptosis and necrosis. Besides, NETs have unique DNA and antimicrobial peptide structures, and antimicrobial activity is among the functions of neutrophils as the first response to inflammation. So, it plays a pivotal role in the pathophysiology of various diseases, especially inflammatory and autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. As a result, it can be effective in the pathogenesis of many diseases, and its pathogenic role can be used as a therapeutic target.

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Introduction

Neutrophils are innate immune phagocytes that play a central role in immune defense. They are equipped with effective antimicrobial that is mainly stored in specialized granules. Because it can also damage host tissue, its establishment is strongly regulated by a variety of strategies, including phagocytosis, production of reactive oxygen species (ROS), degranulation, and release of neutrophil extracellular traps (NET) [1, 2]. The discovery of NET in 2004 opened a modern chapter within immune-mediated microbial murdering. Cooper et al. mentioned that when catastrophically invigorated, neutrophils experience an unused shape of a modified cell passing called NETosis, which decompresses all of their chromatin/DNA, and the resulting structure is discharged within the cytoplasm [3]. During the formation of NETs, the plasma membrane ruptures in a programmed manner. It suggests that the NETs formation involves a suicidal plan morphologically different from other types of cell death, such as apoptosis and necrosis [4]. NETs are specific structures of basket-like DNA with antimicrobial peptides [5]. Antimicrobial activity is among the functions of neutrophils as the first response to inflammation [6]. The neutrophil nucleus loses its shape following stimulation, and the chromatin is decompressed by the peptidyl arginine deiminase 4 (PAD4) enzyme [7]. Then the nucleus membrane and granules are destroyed, and the NET components are mixed. Eventually, the core membrane ruptures, and the NET structure is released. This type of cell death differs from necrosis and apoptosis and is called NETosis [8]. The formation of this structure requires ATPs to organize the microtubule network [9].

One of the most characteristic features of NETosis is the fracture of the nucleus accumbens. This feature distinguishes it from apoptosis and is very reminiscent of the collapse of the nuclear envelope during mitosis in dividing cells [10]. In addition, NET-forming neutrophils lack DNA fragmentation and normal phosphatidylserine exposure to the apoptotic cell death pathway [11]. NET formation and release of mitochondrial DNA do not necessarily lead to neutrophil death or life-shortening [12].

Microorganisms, chemical compounds, and inflammatory mediators, such as Interleukin (IL)-8, complement factor 5a, N-formyl-methionyl-leucyl-phenylalanine, lipopolysaccharide (LPS), and tumor necrosis factor (TNF), as well as pathogens such as *Shigella flexneri*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus pneumonia*, and *Albicans*, can stimulate NET formation, which causes DNA release and neutrophil degranulation [13, 14]. The results show that thick filaments of NET are aggregates of thinner fibers less than 2 nanometers in diameter. In addition, it has been observed that an extensive net is formed in places where thick fibers disintegrate, often attached to spherical structures about 10 nm in diameter. They can combine to form larger masses. The entanglement of these chromatin filaments results in a three-dimensional extracellular structure [15].

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NETs are net-like structures composed of nuclear DNA, histones, and proteins such as neutrophil granule proteins, which include myeloperoxidase (MPO), neutrophil elastase (NE), lactoferrin, and cathepsin G [16, 17].

Steps for the NET formation

NET production may occur based on three paths: 1- slow cell death, 2- vesicular secretion leading to the rapid exit of NETs, 3- the mechanism of the formation of NET from mitochondria DNA (mtDNA) [18]. In the first case, nuclear components are scarce, including type B lamin, histones or poly (ADP-ribose) polymer, and other elements such as cytochrome c, b-actin, and cytoplasmic caspase, 3. However, the common feature of these two types of NET is the presence of two proteins -MPO and NE- associated with the mtDNA [15].

In the acute trauma of environmental tissue, mtDNA and oxidized mtDNA forms the NET and sterile inflammation. MtDNA increases the ROS and expresses the rac family small GTPase 2 (Rac 2), PAD4, and the mtDNA oxidized through cyclic GMP-AMP synthase (cGAS), and toll-like receptor 9 (TLR9) activate neutrophils and forms NET. In general, mtDNA forms NET by starting TLR9, ERK1/2, and p38 MAPK signals [19].

Phorbol-12-myristate-13-acetate (PMA) is directly connected to the protein kinase C, and calcium is released from intracellular reserves and activates the Raf-MEK-ERK path. The nicotinamide adenine dinucleotide phosphate (NADPH) complex is accumulated downstream in the phagosomal membrane and produces active oxygen species. Many NETosis inducers stimulate the signaling of MAP kinase, activate NADPH oxidase (NOX2), and further lead to ROS production, leading to the release of NET and, subsequently, the MPO release of azurophilic granules [20]. It results in the rupture of the granule using a protein complex called "acrosome," which transfers NE to the core [2]. The NE protein serine is mobilized with the MPO and transferred to the core. NE breaks down histones to boost chromatin density [4]. Increasing intracellular calcium levels also activates peptidyl arginine deiminase 4, which reduces the positive charge of histones by the citrullination of these proteins. All of these molecular events reduce chromatin density. As a result, after about 2 hours, the PMA-stimulated neutrophils lose their nucleus heterochromatic regions and the characteristics of nuclear lobules. Then, the cores accumulate and expand. The nuclear cover breaks down into vesicles, granules, and mitochondria. The cytoplasm and the protoplasm are intermixed, and eventually, the cell membrane tears apart and releases cellular content that forms NET in cellular space [21]. This series of events is called suicide.

In contrast, it has been reported that neutrophils can release the whole core or parts of it without breaking the cell membrane. As a result, nuclear cytoplasts will still be able to move phagocyte bacteria, although their crawling pattern will differ from nucleus-containing neutrophils. Since neutrophils survive this form of DNA evacuation, it is called critical NETosis [22].

The formation of the NETs occurs from two general NOX- dependent and NOX- independent routes. NOX2 produces several active oxygen species that activate the NOX-dependent pathway needed to activate ERK, Akt, and P38. In this direction, ERK activation is required (the Raf-

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MEK-ERK path) [23], while it is less activated in the independent pathway of NOX [24]. In the NOX-dependent path, the MPO must form and process the ROS to lead to the NET formation [25]. The NOX- independent pathway occurs due to the mitochondrial ROS and the potassium channel activated with calcium. Akt activation is required in both ways, and the amount of p38 activation is the same. PMA is a factor that plays a role in activating the NET from the NOX-dependent path in cell culture [26]. One of the differentiating factors of NET-based NOX is using an antibody against the histone N terminal tail to detect MOP- histone complexes. In this way, NET-based NOX, the neutron elastases break the N terminals of the core histones during the NET formation [26]. Neutrophil exposure to PMA or LPS can result in NET formation by the NOX-dependent path [27]. In the NOX-independent pathway, activated platelets are agents of NET formation [28]. Autophagy is one of the factors that trigger the formation of NET without ROS, which acts through the Macrophage-inducible C-type lectin [29] (Fig. 1). ROS is needed to release mtDNA in the NET formation process. Recently, it has been reported that ROS production by NADPH oxidase is essential for the viscosity of neutrophils. Also, it has recently been shown that NADPH oxidase produces ROS in neutrophils and regulates actin polymerization through reversible actin glutathione. Actin polymerization plays a vital role in starting ROS production in neutrophils by strengthening the accumulation and activity of NADPH oxidase. Actin and tubulin are glutathione proteins in neutrophils that undergo physiological activation and lead to the NET formation. Cysteine, located at the end of C actin (Cys374), is required for the glutathione of actin-induced ROS, actin polymerization, and NET formation [30]. Various stimuli can induce NET formation. Although it may run through different paths, it is accompanied by a transcription induction. MAPK signaling is essential for the NET formation and activating neutron transcription programs, and the NET formation is independent of molecular synthesis. Therefore, neutron activation involves parallel paths: The path that relies on transcription to produce chemokines and to enhance inflammatory responses, and the latter is independent of transcription, which leads to NET formation and other antimicrobial effects [18, 31].

NET and autoimmune diseases

In addition to NET's role in infectious diseases, such as fungal and parasitic infections, NET formation is involved in autoimmune diseases. To this end, NET's role has been suggested in rheumatoid arthritis (RA) [32], systemic lupus erythematosus (SLE) [33], Antineutrophil cytoplasmic antibody (ANCA) [34], Psoriasis [35], Hashimoto thyroiditis [36], and anti phospholipid syndrome (APS). For instance, one of the clinical manifestations of APS is intravenous and arterial thrombosis; since NETs are implicated in thrombosis, the potential role of NET in APS can be justified.

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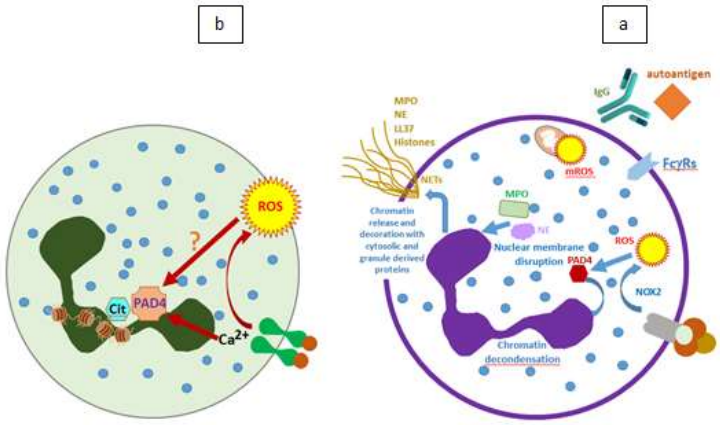


Fig. 1. Mechanisms of NET formation; NOX-dependent path (a) and NOX-independent path (b). PAD4= Peptidyl arginine deiminase 4; NADPH oxidase= Nicotinamide adenine dinucleotide phosphate oxidase; ROS= Reactive oxygen species; MPO= Myeloperoxidase; NETs= Neutrophil extracellular traps; Cit= Citrulline

So far, NET's direct association with Addison's disease, Pernicious anemia, Graves, and Sjogren's syndrome has not been reported [37]. Nonetheless, it is suggested that in patients with Graves treated with Propylthiouracil, this medication affected morphological abnormalities during NET formation and led to ANCA production [38]. In addition, anti-NET antibodies (ANETA) have been detected in Sjogren's syndrome [39]. Many SLE patients have undergone several inactive mutations in DNAse I, which indicate the correlation between autoimmune diseases and disruption of NET clearing processes [40]. It should be noted that limited studies have been conducted on the role of NET in the pathogenesis of multiple sclerosis (MS) and Hashimoto thyroiditis, and further studies are required.

NETs and their role in SLE

SLE is an inflammatory and autoimmune disease that affects different body organs and is associated with excessive levels of autoantibodies [41]. Factors such as functional impairment of lymphocytes and dendrites and anti-nuclear antibodies play a role in the pathogenesis of the disease [42]. The NETs level was studied in skin lesions in various lupus subtypes, such as lupus discoid, acute skin lupus, and lupus panniculitis. It is reported that NETs have contributed to tissue damage in lupus panniculitis. Evidence shows that the NETolytic activity declines in people with lupus. Thus, the level of specific NET indicators, including MPO and cfDNA, rises in the serum of these patients [43, 44]. Hakkim et al. reported that due to DNase1 inhibitors in SLE patients, NETs could not be destroyed, which can exacerbate lupus disease [45]. In patients with lupus, apoptotic particles containing acetylated histones produce accumulation and NET formation, consequently causing lupus nephritis [46].

Role of NETs in RA

RA is a chronic inflammatory disease that is associated with sinusitis inflammation and hyperplasia (swelling), cartilage and bone degradation (deformation), and systemic features, including cardiovascular disorders, pulmonary, Mental and skeletal muscle, which ultimately leads to severe physical disability and early death. Due to the presence of

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autoantibodies such as the rheumatoid factor (RF) and anti-protein antibody [47], RA is considered an autoimmune disease. Studies have shown that RA is a multi-factor disease that age, sex, environmental factors, and genetics could play a role in its pathogenesis. However, its pathogenesis is not fully understood [48]. Various elements in the blood of patients with RA, such as antibodies or immunosuppressive molecules, appear to stimulate NET formation [49]. NET over-formation produces amino acids such as citH2B, citH2B, and citH4 histones. In addition, NET-induced citrullinated vimentin is a central autoimmune factor that stimulates the secretion of proinflammatory cytokines (such as TNF-α and IL-1) and the expression of PAD4 along with the Kapa B nuclear factor ligand (RANKL) activation in the Fibroblast-like synoviocyte [50]. Fibroblast-like synoviocyte, the key inflammation agent in RA, produces several cytokines that damage the joints [51].

Furthermore, the proposed citrullinated antigens actuate antigen-based immune system reactions and lead to the generation of anti-NET autoantibodies. Subsequently, unremitting immune system irritation will exist for a long time. Anti-NET RA (rm Abs) monoclonal antibodies delivered from B- synovial CD19+ cells collected from RA patients are always associated with the NET arrangement. NET antigen resistance depends on the physical hypermutation within the variable Heavy chain and variable Light of the B-synovial cell receptor. In expansion, the glycosylation joined to the Fab-N decides the reactivity of antibodies [52]. The RF, anti-citrullinated protein antibodies, and other antibodies in blood or sinusoidal fluid strongly trigger NET formation in RA [53]. IgG or IgM collected from peripheral blood or synovial fluid in RA patients could trigger more NET antibody production than IgM and IgG collected from healthy people [54].

Hashimoto thyroiditis

Hashimoto thyroiditis, or chronic lymphocyte thyroiditis or autoimmune thyroiditis, is the most common thyroid clinical inflammation, mainly determined by T cells and less by B cells in histology. Among the biochemical characteristics of Hashimoto thyroiditis, antibodies can be found against two major thyroid antigens, including thyroid peroxidase (TPO) and thyroglobulin (TG) [55, 56]. TPO is a membrane protein that syntheses thyroid hormones, such as thyroxin (T4) and triiodothyronine (T3), in the apical membrane of the follicular cells. TG glycoprotein is inside the follicle and is vital in synthesizing thyroid hormones. There is a correlation between the severity of histological thyroiditis and the level of thyroid antibodies in the serum of Hashimoto thyroiditis patients [57].

Furthermore, studies show a significant positive relationship between the concentration of NETs (with the help of the NE and PR3 indicator) and the TG Ab and TPO Ab header in the plasma, which can prove the role of NETs in Hashimoto thyroiditis pathogenesis. One of the most important cytokines in the inflammation process is IL-6. Researchers have considered IL-6 an inducing factor of NETosis in Hashimoto thyroiditis pathogenesis, stimulating and promoting inflammation in the thyroid gland tissue [58]. Although further

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investigation of NETosis's role in Hashimoto thyroiditis pathogenesis is required, it can be expected that in the future, NET can be one of the potential therapeutic mechanisms in patients with Hashimoto thyroiditis.

Multiple Sclerosis (MS)

MS is a chronic inflammatory disorder and central nervous system demyelination. In this disease, an autoimmune response to axon's myelin sheathes leads to axon atrophy and the death of neurons. MS is a heterogeneous disease that is found in two major forms, the extinguishing agent (RMS) and the primary progressive MS, which affects about 85-90% and 10-15% of patients, respectively [59, 60]. PAD4, a member of the pad family, is a cytoplasmic enzyme involved in histones citrullination in NETosis. PAD4 contains a nuclear localization signal (PPAKKST) that does not exist in other PAD enzymes. It has been reported in several studies that the concentration of PAD4 in white central nervous system of MS patients was higher than that of healthy people (control group). These findings highlight the important role of nuclear PAD4 in MS pathogenesis [61-63]. In addition, a high level of IL-8, circulating neutrophils, and NETs in the serum of RMS patients has been observed; Some researchers mention that IL-8 is involved in stimulating the NET formation and prolonging the survival of neutrophils in MS. On the other hand, the increase in neutrophils in these patients indicates effective mechanisms such as degranulation, oxidative explosion, and NET diffusion, which can ultimately exacerbate inflammation and tissue damage [64].

Sjogren's syndrome

Sjogren's syndrome is a systemic autoimmune disease that mainly affects the exocrine glands (mostly salivary and lacrimal glands) and leads to severe dryness of the mucosal surfaces [69]. About 76% of ANETA were observed in the serum of patients with Sjogren's syndrome, indicating the importance of NETs in Sjogren's syndrome [70].

NET and diabetes

Type 1 diabetes (T1D)

T1D is an autoimmune disease that destroys β cells that produce insulin [65]. In patients with T1D, an average decrease in the neutrophil count has been reported with increased circulating protein levels and enzymatic activities of NE and proteinase 3 (PR3). These changes are partly attributed to the strengthening of the NETosis process and increasing NET formation, leading to the release of NE and PR3 into the bloodstream, where NE and PR3 are used as sensitive biomarkers to diagnose type 1 diabetes [66]. Based on experimental findings, physiological beta cell death induces the uptake and activation of B-1a cells, neutrophils, and plasmacytoid dendritic cells (pDCs) into the pancreas. Activated B-1a cells secrete dsDNA-specific IgGs (dsDNA-specific IgGs) that, by stimulating neutrophils, release the Catlesidine-associated antimicrobial peptide to bind to their DNA. Eventually, interferon-α is produced in the pancreatic islets. These studies confirm the association between the existence of NETs and the release of catlesidine-associated antimicrobial peptide. NETs are key in delaying wound healing [67, 68].

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In the mouse model, the NET formation was observed in the islets of Langerhans in the early second week after delivery, while clinical studies in patients with T1D showed an increase in the rate of NETosis and a positive association with circulating NE. It may indicate a key role for neutrophils and NETosis in initiating autoimmunity in the pancreas [67].

Type 2 diabetes mellitus (T2DM)

T2DM could be an unremitting illness characterized by tall blood glucose levels, impeded affront discharge, and affront resistance due to impeded carbohydrate, fat, and protein digestion system. Affront resistance happens for a long time; recently, the onset of T2DM is due to weight, the need for physical inertia, and hereditary inclination [72]. NETosis occurs during inflammation, and patients with T2DM have a low-level chronic inflammation condition [73]. Neutrophil activation is increased in obese and T2DM patients compared with lean individuals. Obesity surgery reduces neutrophil activation in patients to some extent [74]. Insulin signaling is disturbed by nitration of the affront receptor β subunit by MPO and debasement of affront receptor substrate 1 by NE [75]. Neutrophil effects can also be caused by direct interaction with fat cells [76].

NET and inflammatory diseases

Under normal circumstances, neutrophils help tissue degradation by phagocytosing necrotic cells to prevent the uptake of more immune cells, releasing mediators to promote growth and angiogenesis, and producing resolvins and proteins [68]. Neutrophils also play an important role in chronic inflammation. Neutrophils are continuously recruited to the site of chronic inflammation and help by releasing serine proteases, forming NETs, and activating other immune cells during the inflammatory process [71].

NET and nonalcoholic fatty liver (NAFLD)

Chronic liver illness is predominant worldwide and is the foremost common cause of passing and complications related to liver infection. NAFLD incorporates various liver conditions, counting nonalcoholic greasy liver maladies characterized by steatosis and nonalcoholic steatohepatitis (NASH) with irritation, liver cell harm, and fibrosis [77]. Several studies in humans and mice have suggested the role of neutrophils in NAFLD. Infiltration of neutrophils into the liver promotes NASH, and circulating neutrophils are associated with the severity of NASH disease [78, 79]. ROS produced by adsorbed neutrophils activate the hepatic stress apoptosis signal, which regulates kinase 1 and p38, and promotes progression to NASH [80, 81].

NET and inflammatory bowel diseases (IBD)

IBD includes Crohn's disease and ulcerative colitis, which are chronic and recurrent inflammations that damage the structure of the gastrointestinal tract. Clinically, inflammatory bowel disease is characterized by severe diarrhea, bleeding, abdominal pain, and fluid loss due to the underlying inflammatory process [82, 83]. In this disease, neutrophils can act as beneficial agents that promote pathogen clearance and wound healing through IL-22 and NETs or as harmful agents that increase inflammation through PAD4, proteases, and ROS secretion [84]. Neutrophils are the first cells to infiltrate and appear involved in

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epithelial barrier damage, tissue destruction by oxidative and proteolytic damage, and the continuation of inflammation with the release of cytokines and chemokines associated with proinflammatory effects. The dominance of the inflammatory and oxidative environment in IBD provides potential NETosis stimuli such as DAMPs, PAMPs, and cytokines [85]. The role of NET-related compounds in the pathophysiology of IBD includes promoting inflammatory responses by modulating cytokine production and maturation and changes in intestinal epithelial barrier function that lead to increased intestinal permeability. Extracellular matrix (ECM) degradation and potential loss of therapeutic effect of monoclonal antibodies are associated with increased proteolytic activity [86]. NETs are a major cause of initiation and progression of colitis that causes intestinal damage by activating macrophages to secrete proinflammatory cytokines IL-6, TNF-α, and monocyte chemoattractant protein-1, activating platelets and endothelial cells [87].

NET and asthma

Asthma may be an inveterate fiery malady of the aviation route that influences individuals of any race, ethnicity, and age. Patients were partitioned into mild, moderate, and extreme asthma based on the recurrence of side effects, the impact on action and rest, and the alteration in lung work [88]. Due to the lack of established golden standards, asthma diagnosis is mainly based on frequent respiratory symptoms. Asthma has various symptoms, including wheezing, shortness of breath, chest, coughing, and different respiratory airflow restrictions [89]. Studies have shown that asthma is mainly caused by the T helper 2 (Th2) cells, which secrete IL-4, IL-5, and IL-13, leading to airway eosinophilia [90]. Asthma can also be caused by airway neutrophil inflammation, especially in acute, severe, and insensitive to glucocorticoid asthma. According to studies, neutrophils play a role in early asthma and eosinophils in late asthma responses. Pulmonary neutrophil penetration in asthma may reflect a "natural" reaction to pneumonic aggravation, which is activated, for case, by aviation route contaminants or the nearness of possibly pathogenic microbes. On the other hand, inalienable variables related to the patient's socioeconomics (counting age-related changes in neutrophil work) and comorbidities, counting weight, body mass index, and affront resistance, can too influence aviation route neutrophilia [91].

NET and dermatomyositis

Dermatomyositis is an idiopathic provocative myopathy characterized by proximal muscle shortcomings and a typical rash. Interstitial lung disease (ILD) is another distinct clinical feature of dermatomyositis that affects the prognosis of the disease [92, 93]. In addition to causing significant cytotoxicity to endothelial cells and disrupting the differentiation of endothelial progenitor cells into adult endothelial cells, Low-density granulocytes (LDGs) can also increase secretion of type I interferons (IFNs), TNF-a and IFN-γ, which are likely to induce large numbers of neutrophils to produce NET in vivo. Studies have shown a significant increase in the percentage of LDG in peripheral blood mononuclear cells in patients with SLE and diabetic patients with ILD. On the other hand, a

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positive correlation between the percentage of LDG and plasma NET-related markers in diabetic patients with ILD could indicate the important role of LDG in the development of DM-related ILD and NET formation [94, 95].

NET and psoriasis

Psoriasis is an inveterate fiery dermatosis characterized by over-the-top expansion of keratinocytes and silver-white plaques due to expanded cell turnover. Neutrophils separated from the patients' blood appeared more noteworthy helpless to ROS-dependent NETosis than neutrophils separated from healthy individuals [96]. Assist ponders appeared that neutrophil take-up and NETs are vital in psoriasis skin injuries. Centrally pathogenic IL-17, discharged by neutrophils, can increment the neutrophil expression of defensins and LL-37, an antimicrobial peptide of the cathelicidin bunch. These molecules have appeared to advance NETosis and irritation in psoriasis plaques and other dermatopathological illnesses [97, 98].

NET and tumors

NETs were found in different tests of human and creature tumors such as pancreatic, breast, liver, and stomach cancers and around metastatic tumors [99, 100]. The part of NETs in tumor improvement progressively includes altering cancer insusceptibility and the interaction between the resistant framework and cancer cells. NETs play a key administrative part within the tumor microenvironment, such as shaping far-off metastases through the discharge of proteases, specifically lattice metalloproteinases and proinflammatory cytokines [101]. NETs increment the accumulation and expansion of single cancer cells and discharge tumor matrix metalloproteinases and NE, contributing to tumor metastasis, which, through ECM debasement, tumor cells can take off their unique location and relocate to other organs [102]. NETs wake torpid cancer cells. The concept of tumor cell torpidity was included for most common strong cancers, counting breast, prostate, lung, colon, and kidney cancers, as well as melanoma and hematologic malignancies, such as different myeloma, lymphoma, and leukemia. Slow-cycle cancer cells can spread within the early stages, causing cancer to repeat [103]. Different mediators, counting granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, CXC, and CCL3, delivered by tumor or stroke cells, fortify granulopoiesis, discharging neutrophils from the bone marrow and their relocation. In expansion, NETs initiate epithelial transmission to mesenchymal cells in tumor cells. NET proteinases can annihilate the ECM and increment the number of cancer cells. NETs can trap circulating cancer cells in expansion, encouraging metastasis [104]. The affiliation of NETs with coagulation changes in tumor patients has highlighted the significance of NETs in cancer. NETs have appeared to actuate cancer-associated thrombosis in tumors, and a marvel went with by a destitute guess in patients. In expansion, higher levels of plasma citrullinated histone H3 (H3Cit) were watched in progressed cancer patients compared with solid people, and neutrophils in cancer patients appeared higher H3Cit substance than others. In expansion, H3Cit within the plasma of cancer patients is related to NE, MPO, IL-6, and IL-8, which are all NETosis activators [105-107].

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NET recognition laboratory methods

Several methods to detect and then quantify NETs, including immunofluorescence microscopy, enzyme-linked immunosorbent assay (ELISA), flow cytometry, and microfluidic assay, have various advantages and disadvantages. The standard gold method for detecting NETs is immunofluorescence microscopy, which works on co-localizing the most specific NET markers, including complexes of extracellular DNA and neutrophil granular enzymes. This technique employs different fluorescent-labeled antibodies against various components of the NETs, specifically anti-DNA, anti-MPO, anti-NE, and anti-citrullinated histones antibodies. The protein-DNA as mentioned above complexes, such as MPO-DNA and NE-DNA complexes, can also be determined in fluid samples through ELISA that uses a capture antibody specific for the protein component and a dsDNA detection antibody [108, 109]. Nowadays, the application of flow cytometry techniques to detect NETs is expanding. This approach allows for the rapid screening of a large number of samples, it is objective, and the results are not biased by the observer [110]. Similar to what we do in immunofluorescence microscopy, we can use fluorescent-labeled antibodies against key NETs constituents in this assay to identify and quantify NETs. Another approach that is used less commonly in research is the quantifying circulating neutrophil extracellular traps, using a microfluidic device that traps cell-free DNA, in addition to an immuno-fluorescence method that locates neutrophil-specific proteins [111].

The most popular method for detecting NET *in vitro* is microscopic observation, which shows the presence of NETs based on the simultaneous staining and co-localization of neutrophil-derived proteins and extracellular DNA. Since the main base of the NET is DNA, Different colors against DNA, such as Dapi [112, 113], Propidium iodide [114], SYTOX [8], and Hoechst [115], are widely used to visualize NET in immunofluorescence microscopy. However, since histones and several granules, such as myeloperoxidase or elastase, also exist in high NET values, the immunization coloring of these enzymes with the relevant antibodies can help better visualize NET. However, the fluorescence microscope has two main limitations: the observer may bias the results and not allow rapid screening of more cells or samples [116].

In outline agreeing to the substance specified, it can be said that NET plays a part in numerous diseases and can be utilized as one of the restorative objectives, even though nowadays, in a few infections, it is utilized as a helpful objective. In others, it is within the early stages of clinical trials, but there are still more illnesses related to its pathogenesis.

Conflict of Interest

The authors declare no conflict of interest.

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