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Neutrophil Extracellular Traps: Formation and Involvement in Disease Progression

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ABSTRACT

Neutrophils are the forerunner in innate immunity by defending the host organisms against infectious pathogens. During such process, neutrophils reach the site of inflammation/infection and eliminate the pathogens by phagocytosis as well as by forming the neutrophil extracellular traps (NETs). NETs trap and eradicate a number of microbes including bacteria, fungi, protozoa, viruses. NETs consist of DNA which is decorated with histones and granular proteins such as neutrophil elastase (NE), gelatinase, myeloperoxidase. NETosis (a process of NETs formation) is also involved in many inflammatory and autoimmune disorders with a major contribution to acute respiratory distress syndrome, sepsis, cystic fibrosis, periodontitis. Hyper NETosis or ineffective clearance of NETs would likely increase the risk of auto-antibody generation against NETs components and contribution in auto-inflammatory diseases. The purpose of this review is intended to highlight the molecular mechanisms of NETosis and its antimicrobial effect. Furthermore, a current status of NETosis in the pathogenesis of inflammatory and autoimmune disorders has been reviewed for better understanding.

Keywords: Autoimmune disease; Inflammation; Neutrophils; Neutrophil extracellular traps

INTRODUCTION

Neutrophils are the innate immune cells that provide strength to the host defense system. During the infections, neutrophils leave the blood vessels and move to the inflammatory site, where they kill pathogens utilizing several mechanisms such as phagocytosis and degranulation.[1] This process involves recruitment of lysosomes and different classes of granules for releasing proteolytic enzymes and free radical formation.[2,3] Indeed, a study conducted by Robertson et al have demonstrated that neutrophil depletion in mice infected with *Staphylococcus aureus* results in reduced clearance of bacteria.[4] Furthermore, a direct role of neutrophil in fungus killing has been well established.[5] Similarly, a study conducted in neutropenic patients clearly demonstrated the critical role of neutrophils in pathogen killing.[6]

Neutrophils mediate the inflammatory response which is supposed to be a multi-step process where endothelium gets activated by including the circulating neutrophils.[7,8] It migrates towards invaders and eliminates through phagocytosis, it also performs

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generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) into pathogen-containing phagocytic vacuoles.[9] Neutrophil ROS, RNS and also components of granules are very much effective to kill human infective agent in vitro.[2]

A novel mechanism, formation of NETs, to eliminate invading pathogens has been reported.[10] NETs seem to be specifically designed to capture and kill pathogens outside the cell. NETs can be released by neutrophils in a process called NETosis. NETs consist of decondensed chromatin fibres decorated with antimicrobial factors distributed by the granules that kill a variety of microbes. These astronomically immense NETs create a physical barrier to obviate microbial dissemination and augment the levels of antimicrobial molecules. Nuclear DNA fibers form a trap scaffold responsible for keeping all antimicrobial factors at the place of infection and providing their high local concentration. Addition of DNase remarkably diminished the antibacterial activity of NETs and improved the pathogen survival.[11]

Alternative Form of Cell Death

NETs formation is an alternative to death by necrosis or apoptosis.[11,12] The mechanism of NETs formation is clearly distinct from apoptosis and necrosis. Table 1 compares the difference between these modes of deaths.

Mechanism of NETs

Neutrophils are stimulated by contact with pathogens like protozoan, bacteria, fungi or their products. After stimulation, the neutrophil chromatin undergoes decondensation. This process is mediated by enzyme stored in the azurophilic granules, NE and MPO. Initially, NE degrades the linker histone protein H1 and the core histone protein, resulting in chromatin decondensation that is enhanced by MPO.[13] Moreover, throughout NETs formation, histone protein H3 undergoes citrullination, a post-translational modification that converts essential arginine residues to citrulline.[14] The citrullination of histone protein is catalyzed by peptidylargininedieminase 4 (PAD4) that is localized in the nucleus of neutrophil.[15]

The most repeatedly used compound to carryout NETosis is PMA. It is an activator which belongs to the protein kinase C (PKC) family of enzymes. PKC activates NADPH oxidase that leads to reactive oxygen species (ROS) generation.[16] PKC mediated phosphorylation of $p47^{phox}$ allows the different subunits of NADPH oxidase to assemble into a functional complex compound which are capable of generating ROS and specifically, superoxide ions.[17] ROS inactivate intracellular caspases to inhibit apoptosis and induce autophagy that promotes the breakdown of cellular membranes throughout NETosis.

Moreover, the role of RIPK1-RIPK3-MLKL signaling in PMA induced NETs formation in gout has also been reported recently.[18] Involvement of PI3Kγ, ERK, PI3Kσ, PKC and Ca^{2+} in *Leishmania*-induced NETs formation has been demonstrated.[19] NETs formation is dependent on NADPH oxidase and MPO mediated ROS generation. This was validated by studies on chronic granulomatous disease (CGD) patients and MPO deficient subjects where NETs were

Table 1. Comparison between apoptosis, necrosis and neutrophil extracellular traps mediated death (NETosis)

Apoptosis	Necrosis	NETosis
DNA fragmentation	No DNA fragmentation	No DNA fragmentation
Cell shrinkage	Cell swelling	Release of granule proteins and chromatin
Phosphatidyl serine (PS) exposure to outer membrane	No PS exposure	No PS exposure
Preservation of organelles and membranes	Disruption of organelles	Mixing of nuclear, organelle and cytoplasmic contents
Does not affect neighbouring cells	Disintegration of cell membrane and release of cellular contents	Excessive NETosis induces cytotoxicity
No inflammation	Inflammatory response	Inflammatory response
Chromatin condensation	Nuclear swelling	Nuclear membrane disintegrate

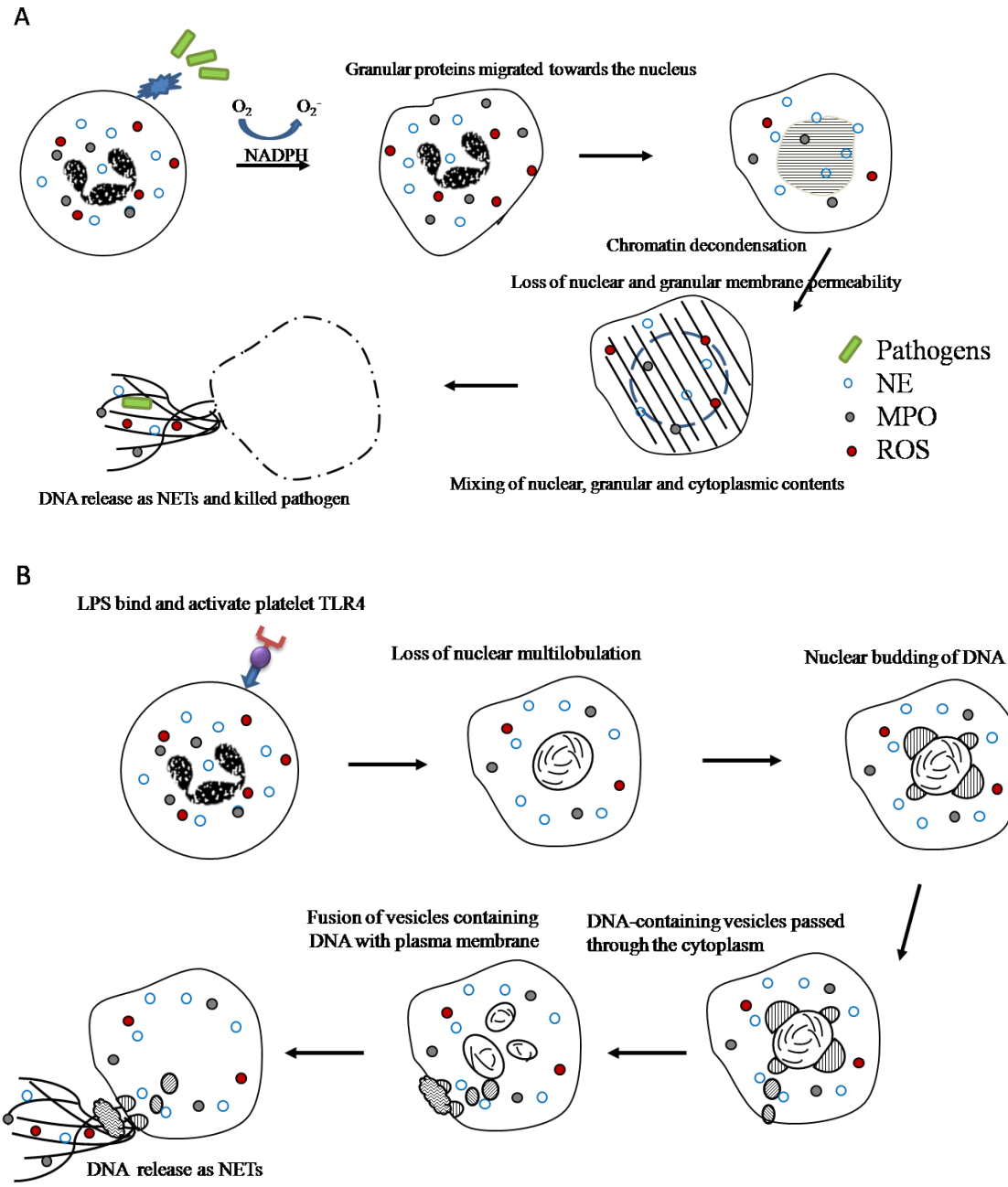

Figure 1. Schematic pathway showing (A) Lytic neutrophil extracellular traps mediated death (NETosis) – This includes loss in polymorph nuclear structure, disintegration of nuclear membrane, decondensation of chromatin, bursting of plasma membrane followed by expulsion of chromatin. (B) Vital neutrophil extracellular traps mediated death (NETosis) – This includes nuclear membrane budding as vesicles containing DNA after interaction of pathogens to the neutrophil membrane receptor followed by fusion of vesicles with membrane and release of DNA containing granule proteins leaving behind active enucleated cell

not formed due to non-functional NADPH oxidase[11] or absence of MPO derived radicals.[17] Two different mechanisms of NETs formation naming lytic NETosis and vital NETosis have been reported (Figure 1)[20] (Table 2). Lytic NETosis has been recognized as a distinct form of active cell death initiated by ligand

binding to neutrophil toll-like receptors, whereas Vital NETosis is lipopolysaccharide (LPS)-stimulated and occurred within the 30 min of involvement of TLR4 on platelets. Neutrophils that released NETs remained structurally intact, therefore, it has given the term vital NETosis.

NETs in Antimicrobial Action

NETs have been shown to decrease the virulence factor of microbes and to restrict their dissemination by forming a physical hurdle. NETs formation is known to be stimulated by several factors like cytokines, bacterial products and clinically relevant pathogen such as Shigella flexneri, Staphylococcus aureus, Salmonella typhimurium, Streptococcus pneumonia and the fungus Candida albicans. NETs formation is also induced by cytokine[21] diacylglycerol (DAG), phorbol-12-myristate-13-acetate (PMA), and nitric oxide.[22] Table 3 depicts the different pathogens which induce NETs upon infection.

While some bacterial pathogen such as P. Aeruginosa,[23] B. Burgoderferi,[24] B. Pseudomallei,[25] S. aureus, S. Pneumonia,[26] N. Meningitides,[27] N. Gonorrhoea,[28] V.cholera,[29] M. Tuberculosis[30] are killed by NETs through the trapping, other appears less susceptible. It has been reported that yeast such as C. albicans[31], fungus such as Aspergillus species[32] and Protozoa such as T. Gondii,[33] P. Falciparum,[34] and Leishmania[19] species capable to induce NETs formation. Some Gram-negative bacteria Acinetobacter baumannii[35] and the fungus Cryptococcus neoformans,[36] are not at all able to induce NETosis. Presence of polysaccharide capsule (glucuronoxylomanan and glucuronoxylomannogalactan) on to C. neoformans confers it to attenuate NETs formation by preventing the signaling pathway. Study involving S. aureus and S. pneumonia mediated NETs formation has highlighted the coordination of innate and adaptive immunity.[37] Furthermore, Braian et al have reported the cooperation between neutrophils and macrophages in the eradication of M. tuberculosis. Neutrophils trap M. tuberculosis in NETs and engulfed by alveolar macrophages. NETs are having a role in antiviral host defense too, where neutrophils are capable of detecting HIV-1 via interaction by toll-like receptors (TLR7 and TLR8). These TLRs recognize viral nucleic acids and induce the generation of reactive oxygen species by MPO-derived oxidants that trigger NET formation and elimination of HIV-1. This response may, however, be counteracted by HIV-infected dendritic cells which release the anti-inflammatory cytokine (IL-10) which, in turn, inhibits NET formation.[38]

Limited information regarding NETs mediated the antifungal effect of neutrophils is known. *C. albicans*infection in human causes 40% of mortality as it is highly opportunistic fungi.[39] Neutrophils release antifungal agent calprotectin which provides protection against host cells. Urban et al have performed an experiment in calprotectin-deficient mice and found it more susceptible against subcutaneous and pulmonary candidiasis as compared to wild- type.[40]

Table 2. Types of neutrophil extracellular traps mediated death (NETosis)

Lytic NETosis	Vital NETosis
Stimulated by physiological inducer like PMA	Stimulated by pathological inducers like bacteria or bacterial product like lipopolysaccharide
Signalling involves activation of protein kinase C and MEK–ERK pathway	Involves activation of TLR4 and platelets followed by direct neutrophil-platelet interaction (Gram-negative infection) and complement receptor 3 and TLR2 (Gram-positive bacteria).
NETs release may take 120 minutes	Rapid process and occur within 30 minutes.
A process which includes unwrapping of chromatin, breakage of nuclear envelope followed by inter-mixing of nucleic acids and granule proteins.	This involves nuclear budding and vesicular release of NETs
It is also called suicidal NETosis as PMNs die after this process	This mechanism allows the PMN to carry out its functions
NADPH oxidase-dependent process	NADPH oxidase-independent phenomenon
ROS dependent activation of cellular pathways leading to translocation of nuclear factor kappa B (NFkB) into nucleus	ROS-independent activation of NFkB

Table 3. Role of neutrophil extracellular traps (NET) in disease pathology

Mechanism	Disease/Condition	Etiology	Type of Cells/ organ involved	References
Hereditary disease	Cystic fibrosis (CF)	Generation of thick sticky mucus	Lung	55
Based on Vaso-occlusion	Coagulation	Damaged blood vessel	RBCs, Platelets	58
	Thrombosis	Reduction in plasminogen activators	RBCs, Platelets	59
Based on Auto-antibody	Periodontitis	Bacterial plaque	Tooth, gingal cavity	66
	ANCA-associated small-vessel vasculitis	Damaged to wall of the vessels	Monocyte, Neutrophil	71
	Systemic lupus erythematosus (SLE)	Destruction of diffuse connective tissues	Skin	79
	Rheumatoid arthritis (RA)	Destruction of immune cells	Bone	86
Based on inflammation	Diabetes	B-cells dysfunction and insulin resistance	Tissue cell	94

Escaping the NETs

Pathogens have evolved different mechanisms to counteract NETs. Most of the Gram-positive bacterial pathogens express DNases for escaping NETs. S. pneumoniae utilize DNase to degrade the DNA which is the ultimate strength of NETs, thereby promoting virulence.[41,42] Furthermore, it has been reported that thermonuclease (Nuc) expressed by gene nuc in Neisseria gonorrhoeae degrades NETs to escape N. gonorrhoeae from killing by neutrophils.[43]

Streptococcus suis is a major swine pathogen and occasionally threatens human health has been shown to induce NETs. These S. suis serotype 2 strains contain polysaccharide capsule over their surface help in resisting phagocytic as well as NETs mediated killing.[44] Morita C et al have discovered SWAN (streptococcal wall-anchored nuclease), a unique cell surface nuclease anchored in the cell membrane of S. sanguinis and investigated its contribution against NETs. Furthermore, deletion of SWAN gene greatly affected the resistivity of S. sanguinis. Moreover, over-expression of SWAN gene in bacterium Lactococcus lactis strain confer more resistant towards NET killing.[45] In other study conducted by Guimaraes-Costa et al have shown that Leishmania infantum can escape NETs mediated killing by 3'-nucleotidase/ nuclease activity.[46] Candida parapsilosis, a fungal pathogen causing disease in premature neonates have been shown to escape NETs through internalization by human endothelial cells via actin polymerization mediated by neuronal Wiskott-Aldrich syndrome Protein (N-WASP).[47]

Interestingly, NETs and DNases are also having the role in mammalian reproduction.[48] During insemination, neutrophil starts migrating into the reproductive tract of female and trap the less motile spermatozoa along with microbial contaminants. However, neutrophils entrap excess of spermatozoa and make infertile. Seminal plasma containing DNases dismantle NETs and free entrapped spermatozoa thus improve the fertility of equestrian and swine spermatozoa.[49]

Implications of NETs in Diseases

Many facts revealed within the past decade demonstrate a negative result of NETs. Many inflammatory processes are delineated with NETosis taking part in a key role as a negative regulator. These processes include lung diseases such as acute respiratory distress syndrome (ARDS), transfusion-related acute lung injury (TRALI), sepsis and cystic fibrosis.[50] Keshari et al, have reported that NO-mediated generation of NETs was capable of bacterial killing, induces a release of pro-inflammatory cytokines hence exhibit inflammatory potential.[51] It has been assumed that NETs formation could play an important role in the progression of autoimmune disorders.[52] Examples of few diseases are mentioned below (Table 3) where involvements of NETs have been well documented.[53]

Cystic Fibrosis (CF)

A hereditary disease aggravated by severe disorders of the respiratory system and gastrointestinal tract. The

principal cause of this disease is a mutation in the CF transmembrane conductance regulator (CFTR) gene which codes for phosphorylation and nucleotide-activated anion channel, results in abnormal Cl^- and bicarbonate transport across the epithelial layer and induce high mucous viscosity.[54] Immune signalling is affected by dysfunction of CFTR. Activation of the toll-like receptor adaptor molecule (Trif) contributes to the acute inflammatory response. TLR4 signals through MyD88 at the cell surface and activates Trif dependent pathways. Trif signalling interfere with NF-kB inflammatory response; constitute activation of Nf-kB signalling results in increased amount of ROS and it associate with increased IL-6 production lead to reduction in CFTR expression. Another factor is the presence of DNA molecules in the sputum of patients with mucoviscidosis, which correlates with the high concentration of neutrophils and the presence of NETs in the lungs.[55] A therapeutic option for mucoviscidosis is the use of recombinant DNase along with protease to cleave DNA/ histone protein complexes typical for NETs. A study conducted by Skopelja et al found the presence of auto-antibodies of NETs localized proteins (bactericidal permeability-increasing protein and carbamylated proteins) in CF patients. Quantitatively this anti-BPI antibody can serve as a marker of deteriorating lung function in CF patients.[56] Additionally, the mechanism involved in NETs release in CF has been demonstrated recently.[57] In this study, it has been found that motile *Pseudomonas aeruginosa* interacts with neutrophils to induce NETs via its flagella. This has a strong impact on inflammation and lung function. *Pseudomonas aeruginosa* isolated from CF patients have been shown to induce DNA, MPO and 4-hydroxynonenal-protein (inflammatory marker of CF) release from human neutrophils.[23]

Coagulation

Coagulation is a crucial part of hemostasis in which a damaged blood vessel wall is plugged by a platelet and a fibrin-containing clot to prevent the hemorrhage,[58] thereby it provides immunity by reducing the dissemination of microbial infection. However, excessive clotting limits the blood flow and results in the poor supply of glucose and oxygen to vital organs which in turn cause severe ischemia. The coagulation pathway depends on tissue factor (TF) expression and its combination with activated factor VII. TF is expressed on cells surrounding the fibroblast to activate fibrin clotting in case of blood vessel injury. Role of NETs in stimulating thrombosis has been reported.[59] During such a process, neutrophils adhere to the vascular endothelium along with the formation of both arterial and venous blood clots. Consequently, neutrophils release chromatin to form NETs that stimulate subsequent thrombosis.[60] Critical role of platelets aggregation on NETs induced coagulation has been studied. Experiments performed in PAD-4 deficient mice resulted in lower platelet aggregation and improved microvascular perfusion as demonstrated by multicolour confocal intravital microscopy.[61] In contrast, Noubouossie et al, have demonstrated that purified DNA and histone proteins rather than intact human NETs induce coagulation under in vitro.[62] This difference is likely explained by the neutralization of negative charge of DNA by histone DNA interaction in intact NETs. Gould et al reported that thrombin generation in platelet poor plasma is reduced with DNase I however not RNase and dismantling the NET scaffold with DNase increase histone-mediated, platelet-dependent thrombin generation.[63] IL-6 promotes coagulation and increases the generation of thrombin without affecting fibrinolysis.[64] Mutlu et al reported that exposure to particulate matter triggers IL-6 production and results in thrombin formation and reduced clotting times.[65]

Thrombosis

Platelets are the key factor for activating neutrophils, generates NETs in lungs and liver during sepsis. Tobias A Fuchs et al demonstrated that NETs co-localize with fibrin in vitro. NETs closely interact with fibrin strand which is present in the thrombus and influencing thrombus stability.[59] Neutrophil gets activated by platelet via binding to the TREM-1 receptor present on the surface of a neutrophil. It causes oxidative burst and IL-8 releases.[66] Thus more neutrophils reach to the site of inflammation. Secretions of neutrophil NE degrade the proteoglycans and promote platelet adhesion.[67] A study has shown that DNase I is able to prevent thrombosis.[59] NETs with citrullinated histones are useful for organizing thrombi, suggesting the link between NETs and thrombosis.[68] M Jimenez et al have reported that low level of DNase-1 lead to an impaired NET degradation in vitro. Reduce plasma DNase-1 activity leads to accumulation of prothrombic Nets in patients.[69]

Periodontitis

Periodontitis is mediated by host-bacterial interaction, results in non-resolving inflammation leading to loss of alveolar bone and tooth loss. IL-4 involved in the process of inflammation, is closely associated with the pathogenesis of periodontitis through enhancing Th2 cell proliferation, supressing Th1 cell proliferation and down regulating Th1-mediated immune response. *Porphyromonas gingivalis* enters the gingival cavity and attracts neutrophils,[70] which then release NETs to prevent the spread of the pathogen. NET act as a protective shield, preventing bacteria adhering to and colonizing gingival epithelium.[71] However, *P. Gingivalis* can escape from the NETs by releasing DNases that degrade the chromatin and enhance the virulence.[72] Leukotoxin produced by *Aggregatibacter actinomycetemcomitans*, associated with aggressive periodontitis has been shown to induce NETosis.[73] Graves et al reported that hyperactivation of the host response to periodontal pathogens due to excessive production of IL-1 and TNF results in periodontal tissue destruction.[74]

ANCA-Associated Small-Vessel Vasculitis

In small-vessel vasculitis (SVV) arterioles, venules, capillaries along with arteries and veins affected. Presence of anti-neutrophil cytoplasmic antibodies (ANCA) against MPO, proteinase 3 (PR3), lactoferrin, NE and cathepsin G has been observed in SVV patients.[75] In SVV, PAD4 mediated citrullination of histones take place, resulting as NETosis. It has been demonstrated recently that treatment of PAD inhibitor significantly reduced NETs release as well as MPO-ANCA production both in vitro and in vivo.[76] This finding opens new avenues to target NETs formation as a potential therapeutic target in ANCA-associated SVV. In AAV, ANCAs are directed against one of two enzymes found in the azurophilic granules of neutrophils, PR3 or MPO. MPO-ANCA is primarily detected in the sera of patients with microscopic polyangiitis (MPA), while serum PR3-ANCA is a marker of granulomatosis with polyangiitis.[77] However, a study from Wang et al has concluded that circulating levels of NETs cannot be used as a biomarker for diagnosis of AAV patients.[78] There is growing evidence that NETs damage the endothelial cells and activate the complement system that results in vessel inflammation in AAV. Thereby, NETs act as a bridge between the innate and adaptive immune system via production of MPO-ANCA and PR3-ANCA.[79] TNF-α primed neutrophils and monocyte express autoantigens on their surface and permits interaction with circulating antibodies.[80] Falk et al reported that ANCA initiated degranulation is enhanced by TNF-priming. In the process of priming, little amount of MPO is expressed at the cell surface and act together with ANCA and instigate neutrophil activation.[81]

Systemic Lupus Erythematosus (SLE)

A severe form of the auto-immune condition, characterized by the production of auto-antibodies due to the collapse of self-tolerance. The resultant antibodies are produced against nuclear as well as granular proteins of neutrophils. Furthermore, ineffective clearance of dying cells by necrosis, apoptosis, as well as NETosis, also provide antigens against which antibodies are produced.[82] Mechanistically, the role of acetylated histone in enhancing the immune-stimulatory potential of NETs in SLE patients have been demonstrated.[83] Such histone modifications could be employed as a therapeutic target in treating SLE. Furthermore, the presence of NETs in placental intervillous has been detected in pregnant women having lupus or pre-eclampsia (or both).[84] This study reveals the increased rate of abnormal pregnancy outcomes and raises the issue of long-term implications for the developing fetus. Additionally, role of mitochondria in NETs generation has been established in SLE.[85] Mechanistically, mitochondrial ROS induced oxidized DNA to come outside neutrophil and triggers inflammation (type one interferon gamma) in SLE. NETs from SLE patients are more capable of activating plasmacytoid dendritic cells (pDCs), a process accompanied by increased secretion of the pro-inflammatory cytokines like IFN-alpha, TNF-alpha, and IL-6.[83] Lande et al have reported that anti-microbial peptides such as LL-37, HMGB1 are required for NETs to activate pDCs involving histone modification.[86] IL-17 has recently been linked to the pathogenesis of SLE, in which participating in amplification of autoimmune responses by stimulating autoantibody production by B cells.[87] IL-17 secreting cells in lupus skin lesions recruit additional neutrophils and also stimulate endothelial cells to produce IL-8 (chemoattractants) which only drive neutrophils. It increases neutrophil adhesion to the endothelium which enhances neutrophil recruitment to organs.[88] Carmelo Carmona et al reported that anti-matrix

metalloproteinases 9 (MMP-9) Abs were detected in lupus sera and directly bound to NETs. Further, an immune complex containing MMP-9 and anti-MMP-9 Abs triggered Net release and enhance NET-MMP-9 activity,[89] this provides a putative mechanism of enhanced NETosis similar to what described by Lande et al for auto Abs to other protein such as LL-37.[86]

Rheumatoid Arthritis (RA)

An autoimmune disease characterized by inflammation in joints. NETs contribute to the generation of anti-citrullinated protein antibody (ACPA) autoantigens.[90] Neutrophils are the predominant cell types present in synovial fluid of patients with RA in abundance. NETosis takes place at the site of inflammation. NETosis is the source of PAD enzyme and citrullinated proteins like vimentin.[15] Calcium ions have the ability to regulate PAD enzyme activity. Indeed it is reported that Ca^{2+} level is very low in RA patient.[91] Various types of auto-antibodies are found in the serum of RA patients. Khandpur et al found that IL-17a and TNF-α can induce NETs formation in RA neutrophils.[92] These NETs externalize citrullinated auto-antigens and promote the release of immune-stimulatory molecules (IL-6, IL-8, chemokines and adhesion molecules) that aggravate the immune response and increase the severity of the disease. In a study conducted by Pratesi et al where they demonstrated that RA patient's serum reacted with deiminated histone H4, which confirms that NETosis externalize antigens against which auto-antibodies are directed.[90] It is reported that perforin and MAC pathway are active in RA joints and represent two immune-mediated membranolytic pathways with the capacity to citrullinate autoantigens in RA. Unlike the other death process (necrosis, apoptosis), the perforin and MAC pathways activate intracellular PADs and induce hypercitrullination.[93]

Diabetes

Under diabetic conditions, neutrophils generate cytokines and superoxide in large quantity. The hyperglycemia can induce NETs formation in type 2 diabetic patient. Hyperglycemic condition causes oxidative stress[94] and constitutively activates NETosis which affect the normal immunological balance and also creates microvascular complications.[95] It has been demonstrated by Diana and colleague; Pancreatic B-cells directly gets affected by IFNs through inducing cytokine and chemokine.[96] IFN enhances the pancreatic B-cells susceptibility to diabetogenic T-cell attacks to autoimmune diabetes in mice. In human, the presence of IFN-alpha in the pancreas of a patient with type 1 diabetes is also reported by Dotta et al.[97] The diabetic microenvironment may thus favours NETosis.[98] In vitro study revealed that increased glucose level induces NETs formation in isolated neutrophils. Augmented levels of NETs components, NE, and glycated hemoglobin has been detected in the plasma sample of the type 2 diabetes patients.[99] Diabetic condition acts as an inducer for neutrophil activation to overproduce NETs and PAD4 and identify NETs as a key factor to delay the process of wound healing.[100] PAD4 carryout citrullination, which promotes NETs formation by inducing chromatin condensation. So, inhibition of PAD4 activity can reduce histone decondensation and NETs formation in response to ionomycin, an ionophore.[101] An experiment was conducted by Wong et al in which, neutrophil isolated from PAD4 lacking mice are incapable of carrying out citrullination process, chromatin decondensation and NETs generation.[100]

CONCLUSION

In summary, neutrophils release NETs composed of chromatin and granule proteins. During NETosis, neutrophils throw its arsenals to trap the pathogen and kill with the help of free radicals and granule proteins. However, pathogens have evolved escaping mechanism by expressing and releasing DNases. NETs are also involved in a variety of inflammatory and auto-immune disorders which results due to excessive NETs formation. There is much evidence with respect to the involvement of NETs in various diseases which is a ray of hope for treating these diseases by blocking NETs formation. However, the major limitation of this process is that NETs have not been the sole factor in the pathogenesis of diseases. Instead, it involves several mediators like cytokines and ROS/ RNS. Therefore, more investigations are required for understanding the mechanism involved in NETs mediated disease progression which will provide new avenues for the search of a better therapeutic target.

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