A perspective on NETosis in diabetes and periodontal diseases

Valliammai Rajendran, Ashita Uppoor

Abstract:
Neutrophil-mediated immunity is the first host defense response against any infection. Crevicular efflux of neutrophils against bacteria is considered to be a novel defense mechanism in periodontal diseases. As a part of defense mechanism, neutrophils extrude its content and exhibit its antimicrobial activity by forming a web-like structure called neutrophil extracellular trap (NET) and undergo a process of cell death called NETosis. Under physiological conditions, NET production is limited and is balanced with its degradation, whereas NET production is found to be aggravated in chronic systemic inflammatory conditions such as diabetes mellitus and also in periodontal diseases. It is well known that a two-way relationship exists between diabetes mellitus and periodontal diseases. Interference in the process of NETosis might form a link between the two. The aim of this review is to focus on the potential role of NETosis in the pathogenesis of periodontitis and diabetes mellitus.

Key words: Diabetes mellitus, inflammation, NETosis, neutrophils, periodontitis

INTRODUCTION

The foremost host response in periodontal infection is by crevicular clearance of pathogens by polymorphonuclear leukocytes (PMNLs). PMNLs are said to phagocytize the pathogens and thereby disrupt the spread of infection.[1] In recent years, research also demonstrated that these neutrophils form extracellular traps to kill the bacteria by degrading its virulence factors.[2] The mechanism of neutrophil extracellular trap (NET) production is different from phagocytosis in such a way that NET kills the pathogens extracellularly and the latter does it intracellularly. In addition, NETs are formed before the microbes can be phagocytized by PMNLs. The crevicular neutrophils in periodontal infections hardly phagocytize the microbes but form plenty of NETs.[3]

BACKGROUND

Even today, the neutrophil activity against an infection is being studied extensively. One such research by Brinkmann et al. in 2004[4] is remarkable in understanding the function of neutrophils in health and various disease processes. An extracellular mechanism in trapping the pathogens was first described by them. Briefly, on stimulation with interleukin-8 (IL-8) and lipopolysaccharides (LPS), the neutrophils formed extracellular web-like structures with nuclear deoxyribonucleic acid (DNA) chromatin and other granules that disarmed and killed the pathogens. Moreover, these web-like structures were termed as NETs. Experimental shigellosis in animals and spontaneous appendicitis in humans showed an upregulated NET formation, and the authors concluded that NET formation is an active mechanism and it is increased in inflammatory sites. PMNLs lose their viability in forming these traps and undergo cell death. Steinberg and Grinstein termed this process of cell death as NETosis by in 2007.[4]

NEUTROPHIL EXTRACELLULAR TRAPS IN HEALTH

Various signals leading to NETosis include nicotinamide adenine dinucleotide phosphate (NADPH) hydrogen activation and production of reactive oxygen species (ROS), activation of receptors for signal transduction (toll-like receptors and cytokine receptors), and activation of peptidyl arginine deiminase IV (PAD4). After activation, neutrophils undergo citrullination, decondensation of chromatin, and destruction of nuclear envelope. The contents from the nucleus are mixed up with the granules in the cytoplasm to form a complex. The formed chromatin-protein complex comes out of the nucleus.
neutrophil by rupturing the plasma membrane.\textsuperscript{[2,5]} The entire process takes $<10$ min, which is faster than apoptosis.\textsuperscript{[2]}

As mentioned previously, NETs acts as a physical barrier and an innate immune response from the host against the spread of infection. It connects innate and acquired immunity by elevating T-cell responses by decreasing their activation thresholds.\textsuperscript{[4]} These traps contain various anti-bacterial substances such as cathelicidin, myeloperoxidase, elastase, and citrullinated histones. Among all these proteins, elastase is the most significant one as it is essential for chromatin decondensation. In experimental studies, it is shown that animals deficient in elastase were not able to form NETs.\textsuperscript{[7]} Apart from all these actions, granular proteins in NETs make the potentially harmful proteins such as proteases from spreading away and prevent it from destroying the tissue surrounding the inflammation.\textsuperscript{[9]} Under physiological conditions, NETs can also be produced by viable cells and are composed of DNA from the mitochondrial component of the cell as compared to NETs from activated neutrophils which contain DNA from the nucleus. Mitochondrial DNA NET formation results in a faster antimicrobial activity after which the cells are stable and do not undergo cell death,\textsuperscript{[9]} whereas with nuclear DNA, NET formation causes more collateral tissue destruction after which the cell undergoes cell death called NETosis. The timely removal of NETs after their action is over is of prime importance. The mechanism behind this remains unclear. It is said that NETs undergo degradation by DNase from the host followed by subsequent clearance by macrophages by lysosomal degradation.\textsuperscript{[10]} In cases of delayed NET clearance from the extracellular environment, DNA can act as a source of autoantigen resulting in autoimmune diseases such as systemic lupus erythematosus\textsuperscript{[11]} and rheumatoid arthritis.\textsuperscript{[12]}

**NEUTROPHIL EXTRACELLULAR TRAPS IN PERIODONTAL DISEASES**

Recently, the literature shows NET production has a significant role in periodontal infections. Kolaparthy \textit{et al}. highlighted the emerging role of NET formation in periodontal diseases.\textsuperscript{[13]} ROS production may be a link in this relation as it is needed for NET formation and it is shown to be increased in patients with periodontal diseases.\textsuperscript{[3,14]} Various periodontal pathogens such as red and orange complex bacteria are capable of producing DNase against host NETs and upregulate bacterial pathogenesis.\textsuperscript{[15,16]} Furthermore, the obstruction of pathogen-associated molecular patterns and damage-associated molecular patterns in periodontal pocket causes exaggerated NET formation in periodontitis.\textsuperscript{[17]} The effect of NETs in chronic periodontitis has been studied, and it was found that there was an abundance of NETs in supragingival plaque, gingival biopsies, purulent crevicular exudate, and gingival crevicular fluid (GCF). The amount of neutrophil elastase present was proportional to the NET formation. It was concluded by the authors that NET formation and crevicular exudate appear to be a novel mechanism in case of chronic periodontitis.\textsuperscript{[3,14]} NET formation in chronic periodontitis patients was compared with healthy controls in peripheral blood, and NETs were quantified 3 months after scaling and root planing (SRP). No differences were seen in NET production between patients and controls at baseline. However, NET quantification in chronic periodontitis patients was significantly reduced after 3 months of SRP.\textsuperscript{[18]}

On the other hand, mutations in \textit{ELANE} (Neutrophil Elastase, Lyophilized) gene encoding neutrophil elastase enzyme, Papillon–Lefèvre syndrome resulting from mutation of cathepsin C, and neutrophil elastase make the neutrophils unable to form NETs in the presence of an infection and result in aggressive periodontitis.\textsuperscript{[19,20]} This shows that adequate amount of NET production is significant for periodontal health maintenance.

Smokers are shown to produce impaired NETs. With cigarette smoke condensate (CSC), there was a dose-dependent reduction in the formation of NETs in peripheral blood from smokers.\textsuperscript{[21]} It may be due to the inhibition of NADPH activation by CSC. Although it is said that nicotine is a potent inducer of NET formation,\textsuperscript{[22]} CSC and cigarette smoke contain various components other than nicotine which may have an adverse effect in NET production. It is well known that smoking is a risk factor for periodontal diseases. Whether impaired NET formation in smokers makes them prone to periodontal diseases remains unclear.

It is well known that the defense action of neutrophils wanes with age. NET production in healthy older individuals (mean age = 69 years) and healthy young individuals (mean age = 25 years) was compared. They concluded that impaired NET formation is thus a novel defect of innate immunity in older adults but does not appear to contribute to the increased incidence of periodontitis in older adults.\textsuperscript{[23]}

**NEUTROPHIL EXTRACELLULAR TRAPS IN DIABETES MELLITUS**

NET production is increased in certain systemic conditions. It has to be noted that the number of neutrophils in the host tissue should not be too many or too less. A state of homeostasis should be maintained. If not, it will be detrimental. Systemic diseases such as diabetes mellitus, deep vein thrombosis, asthma, chronic obstructive pulmonary disorder, acute respiratory distress syndrome, cystic fibrosis, and Alzheimer’s disease are linked with an exaggerated NET production and collateral tissue damage.\textsuperscript{[24,25]}

Type 2 diabetes is linked with low-grade inflammation with marked increase in pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-alpha which are strong inducers of NETs.\textsuperscript{[26]} The expression of PAD4 enzyme is found to be elevated in participants with type 2 diabetes.\textsuperscript{[27]} NETosis was exactly reproduced in cells exposed to high (25 mM) glucose, and also, there were increased NETosis and release of NETs compared with 5 mM glucose and 25 mM mannitol.\textsuperscript{[28]} The elevated NET production in diabetic population reached the level equal to healthy controls after 6 months of glycemic control.\textsuperscript{[29]} It is shown that constitutive NETs are produced in diabetic individuals, leading to downregulated response to LPS, and it is indicated as one of the potent causes of frequent infections seen in those individuals.\textsuperscript{[30]}

**NEUTROPHIL EXTRACELLULAR TRAPS IN DIABETES MELLITUS AND PERIODONTAL DISEASES**

Although various systemic diseases are associated with periodontal infections, a strong association is found between
periodontal diseases and type 2 diabetes mellitus and it is well understood.[13] Individuals with type 2 diabetes and periodontitis are more prone to periodontal tissue destruction when compared with individuals with only periodontitis. NET formation may play a role in periodontal diseases seen in participants with both type 2 diabetes and periodontitis. There is no evidence to prove this hypothesis. NET formation in both type 2 diabetes and periodontitis has been studied separately. Further studies are needed to find the action of NET in periodontal breakdown in individuals with both type 2 diabetes and periodontitis. It is well known that pus, a neutrophil-rich fluid, is commonly seen in poorly controlled diabetes patients. However, it remains unclear, whether an exaggerated NET production may have a role in the etiopathogenesis of multiple periodontal abscesses in these patients.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Knowing that PMNLs functions as a double-edged sword, various neutrophil-modulating approaches have come up in recent times.[10] Local application 0.2% hyaluronic acid as an adjunct to SRP in periodontitis sites has shown to reduce GCF elastase levels over 6-week period.[12] Modulators such as diphenyleneiodonium (NADPH-oxidase inhibitor), N-acetyl-cysteine and taurine (ROS inhibitors), and ranirestat (aldose reductase inhibitor) are shown to cause reduction in NET release.[33,34]

By understanding the mechanism in which various therapies work, NET modulators may be incorporated in dentifrices or a locally applied agent so that its effects are easily delivered. Hence, NET formation may be fine-tuned to modulate its role in periodontal diseases. Further research and long-term studies are needed in this area in analyzing these promising therapies.

Few questions relating to the impact of NETs in host defense remain incompletely understood. Hence, future studies should focus on (1) NET production and its significance in (a) smokers with periodontal disease, (b) periodontal disease with diabetes mellitus, and (c) smokers with both periodontal disease and diabetes mellitus, (2) exaggerated response of the periodontium in uncontrolled diabetic patients and NETs link, and (3) therapeutic modalities in modulating NET response in favor of periodontal health. This can be achieved by increasing our understanding of the molecular mechanisms behind NET formation, elucidating the regulation of NETosis, and investigating the function of these processes in periodontal diseases.

CONCLUSION

To date, since NETs are discovered, there has been an increasing progress about the defense actions of NETs and its role in pathogenesis. In particular, studying the impact NETs in periodontal diseases has become an area of growing interest. NETs appear to potentially increase the anti-infective role of neutrophils, by increasing the defense activity beyond cell death, thereby ensuring utmost utilization of antimicrobial proteins. On the other hand, NETs could be detrimental to periodontal tissues when produced in excess.[14] Hence, a balance in NET formation has to be maintained for adequate periodontal health. In the field of periodontal medicine, various therapies have been proposed. NET modulation in periodontal diseases may be one of the future therapies requiring further investigations.

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Conflicts of interest
There are no conflicts of interest.

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