Janus-Faced Neutrophil Extracellular Traps in Periodontitis

Ljubomir Vitkov[1,2]*, Dominik Hartl[3], Bernd Minnich[1] and Matthias Hannig[2]

[1] Department of Cell Biology and Physiology, Division of Animal Structure and Function, University of Salzburg, Salzburg, Austria, [2] Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, Saarland University, Homburg, Germany, [3] Department of Paediatrics, Paediatric Infectiology, Immunology and Cystic Fibrosis, Children's Hospital, University of Tübingen, Tübingen, Germany

Periodontitis is characterized by PMN infiltration and formation of neutrophil extracellular traps (NETs). However, their functional role for periodontal health remains complex and partially understood. The main function of NETs appears to be evacuation of dental plaque pathogen-associated molecular patterns. The inability to produce NETs is concomitant with aggressive periodontitis. But in cases with exaggerated NET production, NETs are unable to maintain periodontal health and bystander damages occur. This pathology can be also demonstrated in animal models using lipopolysaccharide as PMN activator. The progress of periodontitis appears to be a consequence of the formation of gingival pockets obstructing the evacuation of both pathogen-associated and damage-associated molecular patterns, which are responsible for the self-perpetuation of inflammation. Thus, besides the pathogenic effects of the periodontal bacteria, the dysregulation of PMN activation appears to play a main role in the periodontal pathology. Consequently, modulation of PMN activation might be a useful approach to periodontal therapy.

Keywords: neutrophils, lipopolysaccharide, Papillon–Lefèvre syndrome, chronic granulomatous disease, bystander damages, NETosis

INTRODUCTION

As in other mucosal infections, the host response to the bacteria in periodontitis is characterised by the mucosal efflux of PMNs (1–3). The PMNs influx into the crevice appears to be the first line of defence against plaque bacteria (4). The crevicular PMNs barely phagocytise (5–8), but abundantly form neutrophil extracellular traps (NETs) (4, 8). NETs are an innate immunity defence mechanism chiefly responsible for preventing the bacterial dissemination (9). They are extracellular web-like fibres generated by activated PMNs and are largely composed of nuclear constituents that disarm and kill bacteria extracellularly. NETs have a DNA backbone, but also contain many bactericidal substances, such as histones, human neutrophil elastase (NE), lysozyme, bactericidal permeability-increasing protein, human peptidoglycan-recognition protein S, and other PMN proteins (9–12). NETs bind Gram-positive as well as Gram-negative bacteria, immobilise them, and thus prevent the colonisation of new host surfaces (9). However, NETs can also be triggered by non-infectious agents (9, 13), placental microparticles (13), and inorganic implants (14) and can be harmful for the host (15–22). The capability of NETs to prevent bacterial spreading or to cause bystander damages makes it difficult to comprehend the role of NETs in periodontitis and their impact on the periodontitis pathology also remains elusive.

MINI-REVIEW

Are NETs Beneficial for Periodontal Health?

Analysing the co-occurrence of periodontitis in patients with both known PMN and NETosis deficiencies may help understand the NET impact of NETs on periodontitis.

Papillon–Lefèvre syndrome (PLS) is an autosomal recessive disorder characterised by palmoplantar keratosis and aggressive periodontitis. PLS results from mutations that inactivate cysteine protease cathepsin C (23), which processes various serine proteases including NE, which is an integral structural part of NETs (24, 25). Patients with PLS are either unable to form NETs or produced them in markedly reduced quantities (26, 27). Likewise, inhibitors of NE proteolytic activity, such as small β-lactam-based, cell-permeable NE inhibitors, block the NET release in neutrophils derived from healthy volunteers (25). In addition, the exogenous human secretory leucocyte protease inhibitor markedly inhibits NET formation in human neutrophils (28). The concomitance of aggressive periodontitis and the inability to form NETs suggest the indispensability of NETs for maintaining periodontal health (**Figure 1A**). Similarly, mutations in ELANE gene encoding NE are associated with aggressive periodontitis in the majority of patients with such mutations (29). Quite recently, the inability to form NETs has been reported for ELANE mutations (30).

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency affecting the innate immune system, caused by mutations in any one of four genes encoding the subunits of the superoxide generating phagocyte NADPH oxidase, resulting in an absence or very low levels of enzyme activity (31). However, periodontitis appears to be occasional in CGD patients. Only isolated cases of periodontitis have been reported in CGD patients (32–34). A survey on 368 CGD patients reported merely nine cases of gingivitis or periodontitis (35). Individuals with inherited deficient NADPH oxidase activity, i.e., CGD patients, are capable of inducing NETosis *via* a NADPH oxidase-independent pathway; either *via* an ROS-dependent mechanism utilising ROS from other sources (36) or an ROS-independent mechanism (37). Many trigger mechanisms could be responsible for NADPH oxidase-independent NETosis in CGD patients.

Thus, NADPH-oxidase-independent NETosis is stimulated by higher doses of hepoxilin A3 (38). Another possibility of CGD PMNs to produce NETs in the crevice is to utilise mitochondrial ROS (39), or other sources, e.g., ROS produced by plaque bacteria as *Streptococcus sanguinis* and *Streptococcus oralis* (40, 41). Further, *Candida albicans* (42) triggers ROS-independent NETosis as well as *Staphylococcus aureus* ROS-independent (43) and oxidant-independent NETosis (44). The fact that CGD patients are not disposed to periodontitis suggests that the oxidative burst does not appear to play a crucial role in maintaining the periodontal health, but NETs constitute the main defence. The main function of NETs appears to be that of shielding the gingiva and clearing bacteria, and their metabolic products, out of the crevice.

The ability of the major periodontal pathogens, i.e., those of red and orange complex, to produce deoxyribonucleases (45) suggests the importance of NETs for the host defence. It has been shown that extracellular nucleases enable periodontal pathogens to degrade the host NETs, leading to increased pathogenicity (46) (**Figure 1B**). Although the bacterial nucleases do not affect the NET proteases, the latter alone are not able to provide sufficient protection against periodontal pathogens.

The inability of patients with PLS and most of those with ELANE mutations to form NETs is concomitant with aggressive periodontitis. The ability of CGD patients to form oxidase-independent NETs is a possible explanation for the rarity of periodontitis in these patients. The most aggressive periodontal pathogens produce DNases to degrade NETs. In sum, the NET deficiency paired with aggressive periodontitis indicates the indispensability of NET for maintaining the periodontal health.

Can NETs Be Harmful in Periodontitis?

The lipopolysaccharide (LPS) component of the cell wall of Gram-negative bacteria is an important pathogen-associated molecular pattern (PAMP) that triggers an innate immune response mainly
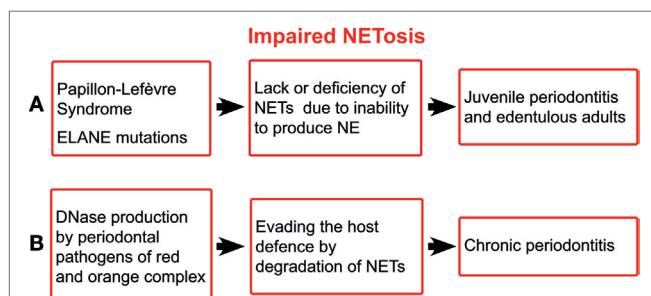

FIGURE 1 | Schematic illustration of impaired NETosis in periodontitis. ELANE, the gene encoding neutrophil elastase; NETs, neutrophil extracellular traps; NE, neutrophil elastase.
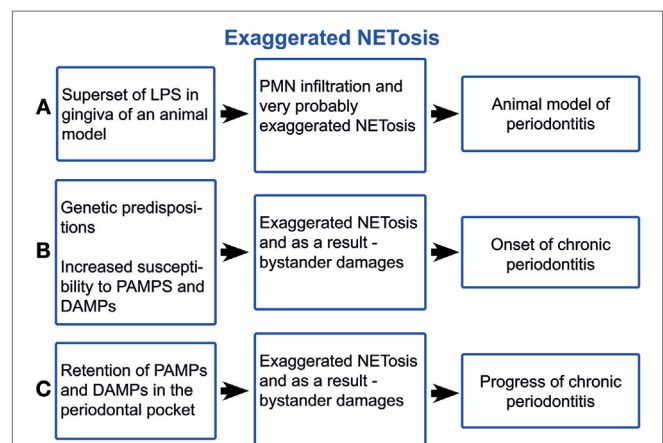

FIGURE 2 | Schematic illustration of exaggerated NETosis in periodontitis. LPS, lipopolysaccharide; PMNs, polymorphonuclear neutrophil leukocytes; PAMPs, pathogen-associated molecular pattern; DAMPs, damage-associated molecular patterns.

through the activation of the toll-like receptor 4. LPS is a potent inducer of NETs (9). The supernatant of dental plaque also triggers NETosis (47). Even elevated blood plasma LPS levels have been registered in aggressive periodontitis (48) (**Figure 2A**). A LPS injection into the gingival tissues is a model for examining how the innate immune response to this bacterial component induces experimental periodontitis (49, 50). Histopathologically, this model is similar to other periodontitis models and to the periodontitis in humans, characterised by increased infiltration of leucocytes, higher levels of pro-inflammatory cytokines, collagen degradation, and alveolar bone resorption. Typically, a defined amount of purified bacterial LPS suspended into small microvolumes (1–6 µl) is injected into the gingival tissues surrounding the posterior teeth of either mice or rats (51). LPS and other plaque PAMPs as well as damage-associated molecular patterns (DAMPs) activate the endothelial cells (ECs), due to the insignificant distance between high endothelial venules (HEVs) and the crevice (52, 53). Alveolar bone loss has been induced by injections of LPS from various microorganisms, including *Escherichia coli*, *Aggregatibacter actinomycetemcomitans*, and *Salmonella typhimurium* (51). LPS-activated ECs become leaky, as shown in the acute lung injury (54), and trigger PMN transmigration. After transmigration across the HEVs, PMNs are attracted to the crevice by PAMPs and DAMPs. LPS-stimulated PMNs selectively secrete IL8, MIP1β, and TNFα (55), which maintain EC activation. Thus, a vicious circle of PMN/HEV mutual paracrine activation may yield an exaggerated PMN response damaging the periodontal tissues. Unquestionably, the LPS effect is not restricted to HEV and PMN activations but affects the entire immunity. Thus, PMN infiltration of gingiva, PMN influx into the crevice, and subsequent NETosis is a crucial feature of periodontitis, which is an exaggerated response to the non-infectious LPS challenge. Nonetheless, PMN efflux cannot be separated from the capillaries and neither can NETosis from the PMN activation, as NETs are just a developmental stage of PMNs. The lack of resolution signals warrants the periodontal inflammation (56). The systemic effects of NETs in periodontal disease may contribute to the body's overall inflammatory burden, worsening conditions such as diabetes mellitus, obstructive pulmonary disease, and atherosclerosis (56–60). Further, periodontitis-derived citrullinated histones (8, 61) may trigger autoimmunity, especially in rheumatoid arthritis.

What Underlies NET Dysregulation in Periodontitis?

Genetic predispositions appear to be crucial for both the onset and the progression of periodontitis (62, 63). Chronic periodontitis occurs when untreated gingivitis progresses to the loss of the gingiva, bone, and ligament, which creates the deep periodontal "pockets" that are a hallmark of the disease (63). The pocket extends the evacuation route of the crevicular fluid, which is the blood ultra-filtrate continuously secreted in the periodontal crevice (64). NETs form a three-dimensional network entangling the particles within the crevice, notably disseminated bacteria, desquamated epithelial cells, cell debris, and fragments of biofilm matrix (4). This network is flushed out by the crevicular fluid outflow. Concomitantly with deepening the periodontal pocket,

morphological changes of the pocket epithelium take place, primarily the inflammatory papillary hyperplasia. As a result, many narrow chasms between the papillae are formed, they are filled with partially and completely exfoliated epithelial cells, which cannot be efficiently flushed out by the crevicular fluid outflow (65), i.e., the pocket obstructs the evacuation of PAMPs and DAMPs out of the crevice (**Figure 2B**). The exaggerated NET formation causes viscosity rise (66, 67) of crevicular fluid and as a result obstruction of PAMP and DAMP evacuation. Further, NET formation is directly induced by many oral bacteria from the dental plaque (41, 47, 68, 69), neutrophil pro-inflammatory chemokines (9, 13, 70), and neutrophil-produced ROS (24). After surgery (71, 72), healing is achieved through the formation of a long junctional epithelium or a new connective tissue attachment to the previously diseased root surface, i.e., through removing the pocket obstruction of the PAMP and DAPM clearing. Thus, periodontitis occurs, given genetic susceptibility (62, 63), as consequence of the exaggerated host response to PAMP and DAPM, as the case of experimental LPS-induced periodontitis is (**Figure 2C**). This self-perpetuating periodontal inflammation has many common characteristics with the chronic obstructive pulmonary disease. Both diseases are characterised by heavy PMN infiltration and NETosis (73, 74), obstruction of PAMP, and DAMP evacuation and aggravation through smoking, as cigarette smoke induces NETs (75).

In cases with exaggerated production of NETs, modulation of PMN activation and NET triggering might be a helpful approach for periodontitis treatment. A broad spectrum of antioxidative substances such as flavonoids, vitamin C, 5-aminosalicylic acid, and N-acetyl-L-cysteine significantly inhibit the formation of ROS-dependent NETs (76). In addition, LPS effects can be reduced by gallic acid and thereby also NETosis (77). In view of the fact that some of these substances are innoxious, they might be applied topically, e.g., as dentifrice or in cases of exacerbations instilled into periodontal pockets. Indeed, further investigations are needed to estimate such possibilities.

CONCLUSION

The inability of patients with PLS and most of those with ELANE mutations to form NETs indicates the role of NETs for maintaining periodontal health. The periodontal pocket formation causes clearance obstruction of PAMPs and DAMPs. The sustained PAMP and DAMP challenge triggers the exaggerated NETosis, which causes bystander damages and the disease progress. Once formed, the periodontal pocket boosts the progress of periodontitis. Modulation of exaggerated NET production by topical application of NET inhibitors might be a possible approach for prevention and treatment of periodontitis.

AUTHOR CONTRIBUTIONS

LV designed the study and drafted the manuscript. LV, DH, BM, and MH critically commented on the paper, contributed towards and approved the final manuscript.

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