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Neutrophils Functions in an Inflammatory Response of Periodontal Diseases

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Abstract: ***Background*:** Periodontal disease refers to any condition that affects the soft tissues, bones, cementum and ligaments that support teeth. Neutrophils in saliva are known to mediate tissue destruction in inflammatory diseases and kill pathogenic microbes through a cell death process known as NETosis. In the realm of periodontal research, the current standard for diagnosis involves the identification of reliable biomarkers for neutrophil mediators. These biomarkers can be utilized to diagnose periodontal disease effectively. ***The Aim***: of this review is to outline the role of peptides neutrophils in periodontal disease. ***Methodology*:** in order to perform this study, relevant publications were searched for using keywords such as": periodontal disease, neutrophils, NETosis, inflammation, immune response." in the academic databases, Web of Science, PubMed, Scopus and Google Scholar. ***Conclusion*:** neutrophils play an important role in the response of inflammation in the periodontal disease.

Keywords: Periodontal Disease, Neutrophils, NETosis, Inflammation, Immune Response.

Review Paper

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INTRODUCTION

Periodontal disease is the second most prevalent disease worldwide which continues to be a serious and contemporary public health issue [1]. Periodontal disease starts with bacterial homeostasis being upset, which causes a rise in in colonization by periodontal pathogenic microorganisms and triggering an inflammatory reaction in the host, this may result in the destruction of tissue [2, 3]. The host immune response is triggered by periodontal bacteria, resulting in the release of cytokines and inflammatory mediators into the periodontal tissues, ultimately leading to periodontal breakdown [4-6]. These host mediators directly or indirectly participate in the damage of periodontal tissue damage and specifically in the resorption of bone. *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* are among the periodontal pathogenic bacteria found in the sub-gingival pockets of periodontitis patients. These bacteria are responsible for the development and progression of periodontitis. In particular, the red complexes consisted of the following extremely harmful species: *P. gingivalis*, *T. forsythensis*, and *T. denticola* [7, 8]. Gingivitis is the mild and the curable type of periodontal disease. Eventually, it often develops into periodontitis. Periodontitis are infectious diseases characterized by immune-mediated destruction of periodontal supporting tissues and tooth loss [9, 10]. The pathogenesis of this condition has not been fully established, the etiology and pathogenesis of periodontal disease are influenced by numerous causes. It is thought that environmental, microbial, and host genetic factors can influence how the disease progresses [11]. Neutrophils also known as polymorphonuclears (PMNs) are the most prevalent leukocytes, whose main function as professional phagocytes with antimicrobial properties is to eliminate extracellular infections. Phagocytic cell types such as neutrophils and macrophages work in concert with other cells to efficiently connect the innate and adaptive arms of the immune response, aiding in tissue healing and inflammatory resolution. Neutrophils are thought to be the primary protective cell type in the periodontal tissues and are widely distributed throughout the gingival crevice and epithelium. According to the

histopathology of periodontal diseases, neutrophils create a "wall" between the pathogen-rich tooth plaque and the junctional epithelium that serves as a cohesive phagocytic apparatus and a strong antimicrobial secretory structure.

Nevertheless, neutrophil defense is not free and is always viewed as a two-edged sword because excessive neutrophil activity can harm tissue and increase the severity and extent of inflammatory periodontal disorders. Neutrophils are essential for preventing bacterial infections. The effector immune cells that activate the antimicrobial defense in the gingiva are neutrophils, and they are the first line of defense against bacterial invasion [12, 13]. As a defense against the inflammatory mediator that the microorganisms secrete, neutrophils carry out phagocytosis and chemotaxis, and they eradicate the microorganisms by releasing human antimicrobial peptides (AMPs) as a non-oxidative antibacterial method [14]. PMNs are essential for the host's defense because they phagocytose invasive pathogens, release reactive oxygen species (ROS), degranulate both inside and outside the cell, and form neutrophil extracellular taps (NETs) [15]. Azurophilic is one of the three primary granules that make up neutrophils which contain alpha-defensin released from gingival crevicular fluid in saliva as an antibacterial defense. Alpha-defensins plays a protective function against oral pathogen bacteria by regulating the development of biofilms, which successfully stops oral infections from happening and protects the tooth structure [16].

Periodontal Diseases

Periodontal disease is a major public health problem due to its high prevalence worldwide. It is an inflammatory condition that associated with bacterial infection [17]. Microbial dental plaque, which accumulates in the gingival sulcus region and activate an inflammatory response initiates periodontal disease, like any common multifactorial disease, genetic and environmental risk factors affect the initiation and progression of periodontal disease [18]. The biological complexity of periodontitis in human is highly comparable to other chronic immune disorders because of multiple factors determine the resultant immune response. Generally, periodontal diseases are categorized into two groups, namely gingivitis and periodontitis [19, 20]. Gingivitis and periodontitis are the two popular types of periodontal disorders. Gingivitis, a steadier form, it is a reversible inflammatory status of the soft tissue around the teeth (gingiva) with no involvement of attachment structure, while periodontitis involves the deeper structure of periodontal tissues leading to loss of attachment with the devastation of gingiva, cementum, periodontal ligament and alveolar bone [21, 22]. Unfortunately, the tissue destruction that happen is irreversible and it is commonly without symptom till loosing teeth, during this period it may have been outspread significantly. Thus, it is distinguished by being extremely hidden, still prevalent, chronic inflammatory disorder which negatively influenced on numerous aspects of life quality [23, 24].

The recent classification of periodontal disease is founded on their severity (slight, moderate, or severe), rate of progression (chronic versus aggressive), extent (localized versus generalized), and localization (i.e., contained within the gingiva, as in gingivitis, or further involving periodontal bone loss, as in periodontitis [22]. Furthermore, the classification of periodontitis has been repeatedly modified. The workshop in (2018) stated that, according to the available knowledge on pathophysiology of periodontal disease, there are three types of periodontitis: necrotizing periodontitis, periodontitis as a sign of a systemic illness, and the types of the disease that used to be referred as "chronic" or "aggressive," but are now all grouped together under the general term "periodontitis" [20].

Pathogenesis of Periodontal Inflammation

Pathogenesis of Periodontal (PD) is caused by molecular and physiological alterations that start with microbial dysbiosis, which is a disturbance in the homeostasis of the microbiota brought on by an imbalance in the metabolic activities and functional makeup of the microbial species, in the oral cavities. A dysbiotic microbiome—mainly Gram-negative anaerobic bacteria—, established either in the enamel tooth surfaces or below the gingival margin may trigger innate immunity pathways by chemical stimulation of neighboring cells in the periodontal epithelium (25, 26), this may also trigger the release of inflammatory mediators [via toll-like receptors (TLRs)] by dendritic cells, gingival fibroblasts, and the periodontal ligament in response to bacterial endotoxins [27, 28]. Pro-inflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, IL-8, IL-12, IL-17, and the receptor activator of the nuclear factor kappa B ligand (RANK-L), are expressed by neighboring cells found in the connective tissue and alveolar bone (29). Failure to eliminate the infection results in the release of pro-inflammatory mediators, which activate B and T cells and initiate adaptive immunity. TNF-α is produced by lymphocytes that have more RANK-L+ B cells than T cells infiltrating connective tissue. When combined with RANK-L and IL-17, this TNF-α increases osteoclastogenesis, bone resorption, and clinical attachment loss (CAL) [30, 31]. This is only the initial stage of the delocalization of inflammation [32, 33]. It's probable that the pathogenic processes could spread to other parts of the body, causing inflammation throughout the body. As we just discussed, periodontal infections may have the ability to directly or indirectly encourage the development of non-oral disorders.Through the production of bacterial toxins or the transfer of microbial

products into the bloodstream, oral microbial dysbiosis can directly cause systemic inflammation.The following families of bacteria may be included in the abundance of microbial species: Treponema, Bacteroides, Porphyromonas, Prevotella, Capnocytophaga, Peptostreptococcus, Fusobacterium, Actinobacillus, and Eikenella. At least three microbial species—Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia—have been shown to be closely linked to PD thus far. The term "red complex" is frequently used to describe these bacteria, which are frequently found in a microbial community. But there are other oral bacteria that could also have an impact on the oral microbial habitat [34]. Entamoeba gingivalis and other oral pathogens have recently been identified as important contributors to the initiation and progression of PD [35]. According to recent research, the oral cavity is residence to 500–700 common taxa, which make up the oral microbiota, also known as the oral microbiome. This cavity, with dental plaque being the most prevalent [36]. It has been observed that *Porphyromonas gingivalis* lipopolysaccharide (LPS) contributes to the development of persistent periodontal inflammation and is linked to certain inflammatory processes in a number of systemic disorders [37, 38]. Researches have already talked about how IL-17 signaling contributes to persistent immune responses and unresolved inflammatory states. The development of environmental circumstances that result in microbial dysbiosis has also been linked to IL-17 cascade. Recent research has examined how IL-17 may cause a change in the microenvironment that favours highly pathogenic bacterial environments and can intensify periodontal inflammation [14]. Furthermore, it has been mentioned that IL-23-dependent IL-17 signalling encourages bacterial proliferation, which helps to create the leukocyte adhesion deficit periodontal phenotype, for example. On the other hand, in periodontitis, IL-17 cascade suppression prevents bacterial proliferation [39, 40].

Neutrophils

Neutrophils (also known as neutrocytes or heterophils) are the most abundant type of granulocytes and make up 40% to 70% of all white blood cells in humans. They form an essential part of the innate immune system, with their functions varying in different animals. They are formed from stem cells in the bone marrow and differentiated into subpopulations of neutrophil-killers and neutrophil-cagers. They are short-lived and highly mobile, as they can enter parts of tissue where other cells/molecules cannot [41]. Neutrophils are a type of phagocyte and are normally found in the bloodstream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure [42], neutrophils are one of the first responders of inflammatory cells to migrate toward the site of inflammation. They migrate through the blood vessels and then through interstitial space, following chemical signals such as IL-8 [43], in a process called chemotaxis. They are recruited to the site of injury within minutes following trauma and are the hallmark of acute inflammation, however, due to some pathogens being indigestible, they might not be able to resolve certain infections without the assistance of other types of immune cells [44].

Inflammatory Mediators of Neutrophil

There are at least four different types of granules seen in neutrophils: primary granules, also known as azurophilic granules; secondary granules, also known as specialised granules; tertiary granules; and secretory vesicles. Elastase, myeloperoxidase, cathepsins, and defensins are among the most virulent mediators that are mostly stored in the primary granules. Among the materials found in the secondary and tertiary granules are lactoferrin and matrix metalloprotease 9 (sometimes referred to as gelatinase B) [45]. The presence of human serum albumin in the secretory vesicles of human neutrophils suggests that they contain extracellular fluid originating from plasma membrane endocytosis. When subjected to gradient media centrifugation, secondary and tertiary granules exhibit overlapping contents but can be differentiated by their intrinsic buoyant densities. Granules can only be released when signaling from receptors in the phagosomal or plasma membranes to the cytoplasm activates their migration to the cell membrane for the secretion of their contents via degranulation. Because the neutrophil's tissue-destructive proteases are greatly enriched, this regulatory mechanism is essential [46]. Neutrophils acquire granules and SV sequentially during their maturation in the bone marrow. The ‘targeting by timing’ hypothesis postulates that the synthesis of granule proteins at the time of formation of granule subset would determine granule content [47]. Appropriate timing of messenger ribonucleic acid mRNA expression during granulopoiesis coincides with granule protein distribution in most proteins used to identify granule subsets [48]. However, the discrepancy in the timing of mRNA expression for a minority of proteins and overlaps in granule content among different subsets (e.g., lysozyme in all three granule types) suggest the involvement of mechanisms in addition to the timing of protein synthesis in the regulation of granule content. Primary granules are the earliest to be formed in promyelocytes (hence their name), stain by the dye azure A (i.e., azurophilic). They contain a large number of antimicrobial proteins, including myeloperoxidase MPO, serine proteases (elastase, proteinase 3, cathepsin G, and azurocidin), α-defensins and lysozyme. Azurophilic granule proteases are activated by proteolytic processing prior to incorporation into granules, resulting in a highly toxic readily available

cargo upon release [49]. Proteomic analysis has identified at least 850 proteins associated with azurophilic granules, of which 135 show the highest relative amounts among granules or are exclusively expressed in this granule subset [48]. Azurophilic granules are heterogeneous in their protein content, which may determine their trafficking to the cell surface or fusion with phagosomes. Plasma membrane-targeted azurophilic granules express Rab27 and Slp1/JFC1, whereas primary granules targeted to the phagosome lack these proteins [50].

Intriguingly, a subset of primary granules does not contain α-defensin, while expressing all other granule proteins. Specific (secondary) granules contain lactoferrin and lipocalin (neutrophil gelatinase associated lipocalin NGAL), but no gelatinase granules GG (MMP-9), whereas GG (tertiary) granules contain MMP-9, but lack lactoferrin and lipocalin. Of 1024 proteins associated with specific granules (SG), 111 are maximally expressed in this subset [48]. GG granules express only 30 of 1123 proteins maximally. A hybrid type granule, constituting about two-thirds of the total peroxidase-negative granules, contains lactoferrin, lipocalin, and MMP-9 (51). SV are formed by endocytosis during the band and segmented stages. These vesicles (markers: albumin, CD45, Mac-1/CD11b, and CD13) are enriched in numerous proteins also present in the cell membrane, including phagocytic, chemoattractant and cytokine receptors, adhesion molecules, membrane components of NADPH oxidase, and plasma proteins. Like specific and GG granules, the release of the content of SV is restricted to the plasma membrane [52], and is thought to facilitate neutrophil adherence to the activated vascular endothelial cells (EC), the initial step in neutrophil trafficking into inflamed or injured tissues. Following firm adhesion of neutrophils to the endothelium, chemokine signaling and outside-in signaling through β_2 integrins induce exocytosis of GG and SG [51].

Function of Neutrophils in Periodontitis

The primary leukocytes attracted to the gingival crevice are neutrophils. After leaving the gingival blood vessels, neutrophils move through the gingival junctional epithelium and eventually arrive at the crevice [53]. Neutrophils form a barrier against the bacterial biofilm that is forming at the gingival crevice. It is believed that this neutrophil wall keeps bacteria from penetrating the tissues underneath. Chemokine and adhesion molecule gradients regulate neutrophil migration in the gingiva. Along the route neutrophils take to reach the gingival crevice, gradients of the chemokine's interleukin-8 CXCL8 (IL-8), intercellular adhesion molecule-1 ICAM-1, and E-selectin have been seen [54]. The primary factor influencing the migration of neutrophils to the periodontium in mice was discovered to be the CXC chemokine receptor 2 (CXCR2), which binds to neutrophil-specific chemoattractant like CXCL1 and CXCL2 (the murine counterparts of human IL-8) [55]. To maintain dental health, neutrophils must be present. This is demonstrated by the development of severe types of periodontitis in those with abnormalities in neutrophil production and distribution. Leukocyte adhesion deficiency (LAD), neutropenias, Papillon-Lefèvre syndrome, and Chediak-Higashi syndrome are a few of these rare and congenital abnormalities. Because of their impaired chemotaxis, neutrophils in the Chediak-Higashi and Papillon-Lefèvre syndromes are not appropriately attracted to infection sites. At a relatively young age, patients with these disorders experience significant and fast loss of periodontal bone [56, 57].

The Papillon-Lefèvre syndrome, in particular, is caused by mutations that leave the cysteine protease cathepsin C inactive. This enzyme is responsible for processing a number of serine proteases that are thought to be necessary for antimicrobial defence. Consequently, these patients' neutrophils produced less cathelicidin LL-37, an antibiotic peptide that inhibits *Actinobacillus actinomycetemcomitans* (58) In GCF from Papillon-Lefèvre syndrome patients, LL-37 was completely absent, even though hCAP18, its precursor, was present in high quantities. The increased incidence of *Aggregatibacter actinomycetemcomitans* infection was associated with the absence of LL-37 [59]. These findings suggest a clear connection between the inability to control infections linked to periodontitis and neutrophil function. Furthermore, it was shown that neutrophils isolated from a different patient with Papillon-Lefèvre syndrome did not express the key neutrophil serine proteases, including proteinase 3, cathepsin G, and elastase. Additionally, these cells were unable to respond to ROS and LL-37 by generating neutrophil extracellular traps (NETs). Remarkably, this patient showed no signs of being more vulnerable to bacterial infections. Hence, it is plausible that serine proteases play a pivotal role not only in the production of antimicrobial peptides and the preservation of gingival crevices' inflammatory homeostasis [60). The significance of neutrophils in preserving healthy oral tissues is emphasized by the fact that congenital conditions characterized by deficits in the number of neutrophils and their function are associated with the development of severe forms of periodontitis. Nonetheless, the presence of neutrophils does not always confer protection. Indeed, the severity of lesions in inflamed periodontal tissues is found to be positively correlated with the number of neutrophils present [61], and the destruction of tissue appears to be an unintended consequence of overactive neutrophils [62]. Additionally, chronic inflammation is associated with severe periodontitis, and neutrophils have been demonstrated to be potent modulators of immunological response and inflammation [63]. Therefore, it appears that neutrophils may contribute to periodontal tissue

damage not only through their heightened activity, but also by disrupting the gingival environment. Neutrophils have the capacity to attract Th17 cells, which can, in turn, elicit the recruitment of additional neutrophils [64, 65]. This phenomenon can initiate a pernicious cycle, whereby the inflammatory state endures indefinitely. The validity of this perspective is supported by the observation that, although neutrophils comprise the vast majority of the leukocyte infiltrate in the gingival crevice and periodontal pockets, lymphocytes constitute approximately 40% of the infiltrating leukocytes in the underlying connective tissue of periodontal lesions [65]. Neutrophils assist in defense and homeostasis by phagocytosis, which is the process of ingesting and destroying antigens within cells as well as they capture pathogens through the process of NETs development that involves the release of histone-decorated, decondensed chromatin along with granular contents into the extracellular space [66].

NETs in Periodontal Disease

The main function of NETs are the gingival protection and clearance of bacteria. The immune response in periodontitis begins as an innate immunity and then develops to an acquired immune response when the amount of antibodies increases [67]. NETosis is a cellular event which ends with cell death and frequently damages individuals directly or through the action of autoimmune systems [68]. The fact of significant quantities of NETs remain in the tissue for a long time gives weight to the role played by neutrophils and their enzymes. Furthermore, this gives weight to the theory that NET formation depends on ROS generation, which has been demonstrated to be elevated in periodontitis. An important factor in determining the periodontal health state of a patient may be the neutrophil function in periodontitis [69]. Natural triggers such as inflammatory mediators produced at the site of the lesion, immune complexes, and damage-associated molecular patterns (DAMPs) may trigger the release of NET. NETs are regulated by a variety of signals, including immunoglobulin-G (IgG) and toll-like receptors (TLRs) [70]. NETs are web-like filaments released by active PMNs. They are made up of antimicrobial peptides (AMPs) like histones, human neutrophil elastase (ELA), myeloperoxidase (MPO), and others, and are constituted of extruded de-condensed nuclear or mitochondrial DNA [71]. NET biomarkers may prove to serve as useful diagnostic and prognostic tools, because elevated NETosis is associated with occurrence and progression of periodontal disease [72]. A recent study found that the salivary biomarkers citrullinated histone3, ELA, MPO and calprotectin were increased in cases of periodontitis and were associated with neutrophil enzymes that mediate NETosis and are connected to the onset and progression of periodontitis [41].

CONCLUSIONS

Neutrophils play a crucial regulator in the inflammatory response in periodontal disease, and could be crucial in the development and maintenance of abnormal immunological responses as well as organ damage in these diseases.

Conflicts of Interest: Authors declare that there is no conflict of interest.

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