Neutrophil priming: Implications in periodontal disease

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Abstract:
Periodontal disease is a well-regulated response to bacterial infection directed by the inflammatory cells of the host immune system. The host response to injury or insult is implicated to be a vital feature of the majority of periodontal diseases. The excessive activation of neutrophils plays a role in the pathogenesis in diseases such as acute respiratory distress syndrome, rheumatoid arthritis, and periodontitis by contributing to inflammatory tissue injury. In the recent times, there has been a shift of paradigm from a hypo- to hyper-responsive/primed model of neutrophil dysfunction in periodontal etiopathogenesis. The aim of this review is to outline the mechanisms and effects of neutrophil priming, and thereafter, discuss the current controversy that exists regarding the role of primed neutrophils in periodontal etiopathogenesis.

Key words:
Aggressive periodontitis, chronic periodontitis, hyperresponsive neutrophil, neutrophil priming

Neutrophil activity at site of inflammation and priming

Neutrophils are the most abundant leukocyte in blood and comprise the basic component of the nonspecific immune response. They are the first line of defense, that is, first cells to be recruited to sites of inflammation. These cells have multiple roles in maintaining the body homeostasis which includes phagocytosis, production of reactive oxygen metabolites, and degranulation of cytotoxic proteins. Although the basic function of neutrophils is to limit the pathogen, the release of some of the products into the surrounding areas may cause “friendly fire” damage at sites of inflammation. It is now well accepted that inappropriate or excessive activation of neutrophils may aid in the pathogenesis of a variety of diseases such as the acute respiratory distress syndrome, rheumatoid arthritis and ischemia, and reperfusion injury by contributing to inflammatory tissue injury.

Periodontal disease is a chronic inflammatory response to bacterial infection mediated by the inflammatory cells of the host immune system. The host response to injury or insult is implicated to be a vital feature of the majority of periodontal diseases. The term “primed neutrophil” has started making an increased appearance in the periodontal literature. The aim of this review is to outline the mechanisms and effects of neutrophil priming and thereafter discuss the current controversy that exists regarding the role of primed neutrophils in periodontal etiopathogenesis.

PRIMING: CAUSES AND EFFECTS

Neutrophil priming was described by McPhail et al. in the early 1980s, as “the ability of a...
primary agonist, typically at substimulatory concentration, to influence/enhance superoxide production triggered by a secondary stimulus.” A priming agent is a substance that by prior exposure enhances the response of a neutrophil to an activating stimulus. In a resting normal circulating neutrophil, the micellular capacity is very low when exposed to activating agents. Once exposed to specific priming agents, this capacity is enhanced several folds. These agents do not express the priming function on their own and have to be in contact with the cell for specific period to prime it. A 20-fold increase in the respiratory burst and release of superoxide anions in response to a secretagogue agonist has been observed in a primed cell as opposed to an unprimed cell.

Mechanisms of priming
Two separate mechanisms have been proposed for priming. Rapid priming occurs within minutes of being stimulated. The short duration of response is as a result of transfer and release of preexisting intracellular granules with preformed receptors to the plasma membrane. In this type of priming, there is no active synthesis of proteins, just an increase in the number and affinity of cell surface receptors. Delayed priming takes more time as compared to rapid priming. Here, the priming agent causes an activation of transcription factors which results in the active synthesis of new protein molecules (including receptors and cytokines).

Priming agents
Several substances are released in the body in response to infection, trauma, and hemorrhage which may act as priming agents. Different priming agents require different exposure time to induce maximal priming of a substrate cell [Table 1]. For example, circulating bacterial endotoxin and increased levels of plasma tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 have been proposed as neutrophil priming agents. Other proposed priming agents include bacterial lipopolysaccharides (LPSs), interferon-γ, IL-8, substance-P, and granulocyte macrophage colony-stimulating factor (GM-CSF). The priming agents may either be present in the bloodstream where they are exposed to the systemically circulating neutrophils or may be produced locally in the tissues (e.g., in the periodontal micro-environment) where they can prime the functional responses of locally extravasated neutrophils. The process of extravasation itself may also lead to some amount of priming. Furthermore, neutrophils present locally at a site of inflammation are primed as compared to the circulating neutrophils as a consequence of the interactions that occur with the activated endothelium during the process of extravasation. However, the implications are much greater when the neutrophils are primed before entering the inflamed periodontal sites (i.e., in systemic circulation).

Since several agents are capable of inducing neutrophil priming, the concept that a single signaling pathway causes neutrophil priming is now redundant. Various mechanisms have been proposed which include heterotrimeric GTP-binding proteins (G-proteins), phospholipase C activation, phospholipase A2 activation, and phosphoinositide 3-kinase activity.

Functional consequences of neutrophil priming
Once primed, many changes occur in the response of neutrophils to microbial challenge [Table 2]. First, as a response to priming, the respiratory burst activity is enhanced. It is expressed as the enhancement of the O2− (superoxide anion) response to bacterial formylated peptide N-formylmethionyl-leucyl-phenylalanine. This is also considered as the gold standard priming response [Figure 1]. This increased burst activity releases an excess of reactive radicals which enhance the tissue injury. Second, there is a change noted in the shape and deformability of the neutrophils. This results in a polarization response which may represent frustrated chemotaxis. The priming process causes some changes in the cytoskeletal actin as a result of which the neutrophil loses its deformability. Such neutrophils are called “stiffened” neutrophils and are retained at the site of inflammation for a long time. Their increase in retention time increases the risk of neutrophil-mediated tissue injury.

### Table 1: Various priming agents

| Priming agent          | Exposure time for maximal priming | References |
|------------------------|----------------------------------|------------|
| ATP                    | 15 s                             | [7]        |
| Substance P            | 1 min                            | [8]        |
| PAF                    | 5 min                            | [9]        |
| TNF-α                  | 10 min                           | [10]       |
| Interleukin-8          | 10 min                           | [11]       |
| LPS                    | 120 min                          | [12]       |
| GM-CSF                 | 120 min                          | [13]       |
| Interferon-γ           | 120 min                          | [14]       |

### Table 2: Functional consequences of neutrophil priming

| Effect of priming                     | Consequence leading to increase tissue injury |
|---------------------------------------|-----------------------------------------------|
| Enhanced neutrophil respiratory burst| Tissue damage                                 |
| Change noted in the shape and deformability of the neutrophils | Prolonged retention                          |
| Augments the cell adhesion molecule function and expression | Increased recruitment                        |
| Modest additive effect on elastase and myeloperoxidase release | Tissue damage                                |
| Delay the onset of neutrophil apoptosis | Prolonged lifespan                           |

Figure 1: Enhancement of superoxide production after priming

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influx of neutrophils into the inflamed focus.\textsuperscript{[17]} This increased recruitment to the focus of infection leads may cause increased tissue injury. Fourth, priming has a minor enhancing effect on elastase and myeloperoxidase release.\textsuperscript{[19]} Fifth, some priming agents including bacterial LPS and (GM-CSF) delay the onset of neutrophil apoptosis. This increases the functional lifespan of the neutrophil and thus increases the corresponding tissue injury.\textsuperscript{[19]} Many of these changes including stiffening, delayed onset of apoptosis, and increased recruitment cause more and prolonged retention of neutrophil at the site of injury. This prolonged retention coupled with sustained, prolonged, excessive release of tissue-destructive factors released by primed neutrophils enhances the concurrent tissue injury rather than making the bacterial clearance more effective.

**Depriming**

Neutrophils may have the ability to undergo priming, depriming, and subsequent repriming cycles. This helps to manage neutrophil activities at the sites of inflammation and offers a potential mechanism for modulating the neutrophil response at sites of inflammation.\textsuperscript{[20]}

**ROLE OF NEUTROPHILS IN PERIODONTITIS**

Periodontitis is a chronic inflammatory condition affecting the structures surrounding the tooth. As it progresses, neutrophil extravasation and accumulation at the site of inflammation is seen. The knowledge of central role which the neutrophil plays in the host response fuelled the idea that it may play a crucial role in the progress from periodontal health to disease. The initial reports in this regards reported that conditions such as Chediak–Higashi Syndrome and Lazy leukocyte syndrome were associated with early and severe periodontal infection, bone loss, tooth mobility, and tooth loss. These are disorders with primary neutrophil deficiency. Other conditions associated with secondary neutrophil impairment such as Downs’s syndrome, Papillon–Lefèvre syndrome, and inflammatory bowel disease also demonstrate an increased risk and significant amount of periodontal destruction. Furthermore, disorders directly involving the functioning of neutrophils such as leukocyte adhesion deficiency type I and II demonstrate severe tissue destruction. It has been observed that induced neutropenia and primary neutrophil abnormalities may lead to rapid periodontal infection.\textsuperscript{[21]} These observations highlighted the importance that altered neutrophil function may play in the pathogenesis of periodontal diseases.

These findings paved the way for the inspection of a model for the periodontal pathogenesis based on a disturbance in neutrophil function in an otherwise healthy person. In the beginning, research revolved around defects in neutrophil chemotaxis and phagocytosis (function). Several studies attributed defective chemotaxis, defective phagocytosis, or defective killing by the neutrophils to be central in the pathology of localized aggressive periodontitis.\textsuperscript{[22–24]} This has been suggested to be a genetic or an acquired defect involving a decrease in number or defective structure at the level of surface receptors.\textsuperscript{[25]} This model had several shortcomings such as its inability to validate the role of neutrophils in aggressive periodontitis and explain the rapid rate of loss of periodontal support in aggressive periodontitis. In some contrasting reports, no difference was observed when neutrophil chemotaxis, phagocytosis, superoxide production, or adhesion in patients with aggressive periodontitis was compared to patients with chronic periodontitis and periodontally healthy controls.\textsuperscript{[26]}

**EMERGENCE OF CONCEPT OF PRIMED NEUTROPHILS**

In light of further studies, a shift of paradigm occurred when the responsive of the neutrophils was measured. The concept of primed neutrophils was then introduced to the field of periodontics. The hyperactivity of these cells has been implicated as the responsible factor for the rapid periodontal destruction commonly associated with aggressive periodontitis. Degranulation and subsequent increased release of various enzymes extracellularly is an essential feature of a primed neutrophil.

Several enzymes were found to be present in increased levels in periodontitis patients. Increasing levels of β-glucuronidase activity in the crevicular fluid of patients with healthy, localized aggressive and generalized aggressive periodontitis cases have been observed.\textsuperscript{[27]} Another study found increased gingival crevicular fluid levels of myeloperoxidase in aggressive periodontitis as compared to controls.\textsuperscript{[28]} Increased elastase levels in neutrophil from chronic periodontitis as compared to controls have been observed. The authors have attributed this difference to priming events.\textsuperscript{[29]} These enzymes are proteolytic and may inhibit the host defense mechanisms. Such an increase in these enzymes was thought to be a result of a priming response which enabled a cell to release excess amounts of these enzymes at the site of inflammation.\textsuperscript{[25]}

The increase in the amount of oxidative burst products produced and released from both resting and stimulated cells is the hallmark of a primed neutrophil. Such products include superoxide, hydroxyl radicals, and hydrogen peroxide.\textsuperscript{[30]} These parameters have been studied in the several studies. In a study, no significant difference between the priming between healthy and chronic periodontitis was observed.\textsuperscript{[31]} Another study demonstrated increased Fc gammaR-mediated chemiluminescence of peripheral neutrophils in chronic periodontitis patients as compared to controls.\textsuperscript{[32]} This study found that unstimulated chemiluminescence by neutrophils is more in aggressive periodontitis patients compared to chronic periodontitis patients.\textsuperscript{[33]} Another study reported that neutrophils from chronic periodontitis patients release more reactive oxygen species as compared to controls in both stimulated and unstimulated scenario.\textsuperscript{[34]} Neutrophils exposed to Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans neutrophils from chronic periodontitis patients released significantly more reactive oxygen species when compared patients with aggressive periodontitis.\textsuperscript{[34]}

Poor glycemic control has also been shown to enhance the production of superoxide radical suggesting an underlying priming response.\textsuperscript{[35]} Conflicting results have been obtained stating no change or decrease in oxidative burst products in both chronic and aggressive forms of periodontitis are present. This difference in the results may be attributed to the difference in the methodology, parameters measured, severity of disease, and possibly other genetic/racial/geographic variations.
A question that can be asked is that it is responsible for the rapid and severe tissue destruction in LAgP. From an analysis of the studies on this topic, it can be easily concluded that neutrophils from chronic and aggressive periodontitis are primed as compared to healthy controls. A topic of conflict is whether priming is exclusive to either chronic form or aggressive form. Even though the literature is conflicting, majority of the recent studies show an association of aggressive periodontitis with the presence of a primed neutrophil along with impaired chemotaxis, phagocytosis, and killing. This hypothesis has been supported by various authors. This phenotype results in diminished local immunity attributed to poor response in terms of chemotaxis and phagocytosis. Its primed state causes an increase in the respiratory burst and degranulation and increased amount of β-glucuronidase, myeloperoxidase and other oxidative burst products. This, in turn, leads to excessive oxidative and proteolytic tissue damage. The combination of limited immune response and excess tissue damage may explain the disproportionately large loss of periodontal structure observed in aggressive periodontitis.

Literature gives conflicting evidence, and further studies are required to elaborate the exact role of primed neutrophils in the pathogenesis of various types of periodontal diseases and to understand the intricacies of various cellular pathways involved in the regulation of expression of this hyperresponsive phenotype.

**PRIMED NEUTROPHILS IN CHRONIC AND AGGRESSIVE PERIODONTITIS**

**Origin**

Apart from these clinical observations, the question that is frequently raised is that whether the presence of primed neutrophils in the patients with chronic and aggressive forms of periodontitis is genetic defect or an acquired one.

The chronic inflammatory nature of periodontal diseases causes several inflammatory mediators to be released in the blood. Prolonged contact with one/more of these can sensitize the resting neutrophils and make them primed. Many agents have been suggested as being responsible for priming of neutrophils. These include TNF-α, bacterial LPS, substance P, phospholipase C, phospholipase A2, IL-8, GM-CSF, and interferon γ. These mediators and products of inflammation are present in the inflamed periodontal tissues in abundance. These locally generated mediators may prime and enhance the functional responses of extravasated neutrophils; it is probable that these priming agents may play a pivotal role in the pathogenesis of periodontal tissue destruction. Limited data are available on the possible priming agents and their mechanisms of action in the periodontal microenvironment.

Furthermore, it has been reported that preincubation of neutrophils from various periodontitis patients with *P. gingivalis* and *A. actinomycetemcomitans* causes several fold increase in the production of oxidative burst products as compared to controls. This indicates that apart from other factors, the exposure of neutrophils to the periopathogens may also be enough to induce some amount of Priming. Limited data are available regarding the priming influence of these bacteria, and more studies are needed to shed light on this topic.

Neutrophil priming by inflammatory cytokines in serum has also been proposed in aggressive periodontitis. In this study, an association between increased IL-8 plasma levels was associated with increased respiratory burst products and decreased L-selectin expression in aggressive periodontitis patients. The authors suggested that IL-8 may act as a priming factor in such scenario. Literature supports the increased presence of priming agents in patients with all forms of periodontitis both locally and systemically. This presents a very real possibility that the presence of primed neutrophils in such patients is a consequence of local or systemic exposure to these agents.

On the other hand, there is evidence that suggests that the presence of primed neutrophils in periodontitis patients has a genetic component to it. Various genetic polymorphisms have been linked to the hyperresponsive phenotype in LAgP. In a study, it was found that C242T p22phox nicotinamide adenine dinucleotide phosphate oxidase and FcgR polymorphisms affect the production of superoxide production from neutrophils and may predispose to aggressive periodontitis.

Another study demonstrated the association of three genes including HSF4b (transcription factor), ZI9 (activator of TGF-β), and muskelin that affect neutrophil function with aggressive periodontitis. It has also been observed in a study that the increased chemiluminescence in localized aggressive patients is maintained even after treatment. In another study, the increased oxidative burst was persisting even after periodontal treatment. This finding points toward an inherent component for the presence of primed neutrophils.

The studies supporting the genetic component are few and sporadic. However, there is not enough scientific evidence to favor one theory over the other. Further studies investigating this controversy are required to determine whether the hyperresponsiveness of neutrophils in periodontal diseases is constitutional or the result of peripheral priming due to the disease process.

**Implications in periodontics**

The proposition that primed neutrophils play a vital role in periodontal tissue destruction is an interesting one. More studies are required to validate this hypothesis. If validated, implications for risk, diagnostic, prognostic, and therapeutic markers are possible. The use of a screening test to assess the risk profile for the development of chronic or aggressive periodontitis based on analysis of neutrophil function, if possible, is a distant possibility. It has been previously suggested that diagnostics based on neutrophil function may improve the accuracy of diagnosis of aggressive versus chronic periodontal diseases.

Many attempts have been made regarding the same. This study found that the number of GP110 receptors on the neutrophil surface was significantly reduced in aggressive periodontitis. The authors suggested that this can be used as a marker for the same. However, contradictory reports stating that normal chemotaxis was seen in neutrophils from LAgP patients are also available. Reports regarding this are sporadic, and their large-scale applicability is questionable.

The use of specific genetic markers to distinguish between various forms of periodontitis such as chronic, localized aggressive and generalized aggressive is also a possible
application. Similar markers can be studied for their application as prognostic factors. The presence or absence of a specific genetic marker can affect the prognosis of the individual case. The screening, diagnostic, and prognostic applications are based on the assumption that genetic variations in the neutrophil response may be responsible for the primed neutrophil. However, it is also largely dependent on the discovery of markers which are specific and universal to these diseases. Whether such markers exist is not known. Detailed epidemiological studies in different populations researching various polymorphisms may make this possible.

In therapeutics, the application would be modulation of the neutrophil response. The local destructive actions of primed neutrophils can be restricted at the same time the antimicrobial activity could be kept intact. A desirable therapeutic target would be minimizing the destruction of the host tissues caused by the “friendly fire” by the neutrophils at the same time not hampering the vital antimicrobial role that they play. This has been an eluding goal and has not been completely achieved till date. The most common application in this field has been that of low dose systemic tetracycline derived antibiotics such as doxycycline. Low nonantimicrobial doses of these have been seen to inhibit proteolytic enzymes released by neutrophils, such as matrix metalloproteinase-8. They also act as scavengers for reactive oxygen species and other products of oxidative burst. This may help in limiting the damaging effects of the neutrophils on adjacent tissues. Furthermore, the role of periodontopathogens such as P. gingivalis and A. actinomycetemcomitans in priming the neutrophils should be investigated as a potential target for therapy.

Other newer anti-inflammatory products such as lipoxins and resolvins may target the neutrophils as a modulatory therapy. One such study reported decreased neutrophil migration in response to lipoxins and aspirin stimulated lipoxin. Another study reported no effect of lipoxins on neutrophils derived from patients with LAgP; however, the inhibitory effect of resolving E1 on superoxide generation was seen. Most of the research in this area has been on animal models and has shown the limited effectiveness of such treatment modalities. The “primed neutrophil theory” is still evolving and is being continuously challenged. More long-term studies are required to establish whether any clinical or therapeutic benefit can be achieved from approaches based on this model.

**CONCLUSIONS**

Priming of neutrophils is one of the vital processes which regulate the intensity of response of neutrophils at the site of inflammation. It can also modulate and enhance the neutrophil response at an inflamed site. Neutrophil priming is a relatively new concept, and its role in the pathophysiology of different forms of periodontal diseases is still unclear. With advances in our understanding of neutrophil biology, various intracellular signaling pathways and mechanisms of tissue destruction, greater insights into the periodontal pathogenesis can be gained.

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