

## “Adapting to terminologies involved in host immune response”

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**Abstract:** The innate immunity can sense the exogenous pathogen motifs referred to as pathogen associated molecular patterns (PAMPs), as well as other signals associated with infection, such as host components like endogenous products released from dying or damaged cells were called damage associated molecular patterns (DAMPs). Thus the immune response is induced not only by exogenous microbial infection, but also by endogenous sterile tissue damage and degeneration. Based on the common recognition system and the similar inflammatory response, some authors use the term “alarmins” to categorize endogenous DAMPs as separate from pathogen-derived molecules. In this review the authors attempt to familiarize the periodontal community with the terminologies involved in host immune response.

**Keywords:** alarmins, DAMPs, immunity, PAMPs, periodontal pathology

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### I. Introduction

The immune system is composed of an innate (non-specific) and an adaptive (specific) response. Innate immunity is constitutively present and is mobilized immediately following infection. Innate immunity is termed non-specific because the protective response is the same regardless of the initiating infection. This is in contrast to the adaptive immune system which is slower, responds specifically, and generates an immunological memory. Pattern recognition receptors (PRRs) expressed by both innate immune and non-immune cells are essential for detecting invading pathogens and initiating the innate and adaptive immune response.

The periodontal community is familiar with the pattern recognition receptor- Toll-like receptors (TLRs). In addition to TLRs, there are other PRRs as well. The goal of this topic is to adapt the periodontal community with molecules and terminologies involved in host immune system with particular focus on innate immunity.

### II. Concept and Terminologies

#### 2.1 PAMPS and receptors

Charles Janeway proposed that the innate immune system uses evolutionarily ancient pattern recognition receptors (PRRs) for the detection and identification of invading pathogens.<sup>[1,2]</sup> The innate immunity can discriminate between pathogenic microorganisms and commensals. The immunogenic exogenous/foreign pathogen motifs are referred to as PAMPs, i.e. *pathogen* - (from Greek ‘pathos’ and ‘genēs’: ‘producer of disease’) *associated molecular patterns*.<sup>[2]</sup> Some “exogenous” PAMPs are sensed by the immune system only when they are present at specific locations, such as flagellin, dsRNA (double stranded RNA), and DNA, which have different effects depending on whether they are present extracellularly or within an infected cell, indicating that the immune system initiates responses based not only on the identity of the PAMPs, but on where and under what cellular context they are presented.

The receptors/sensors to recognize PAMPs include Toll-like receptors (TLRs), retinoid acid inducible gene (RIG)-I like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs)<sup>1</sup> and the recently identified cytosolic DNA receptors.<sup>[3]</sup>

#### 2.1.1 Toll like Receptors (TLRs)

Toll-like receptors (TLRs) are a family of type I transmembrane pattern recognition receptors (PRRs) that are expressed by a number of different immune and non-immune cell types including monocytes, macrophages, dendritic cells, neutrophils, B cells, T cells, fibroblasts, endothelial cells, and epithelial cells. TLRs recognize conserved, “exogenous” pathogen-associated molecular patterns (PAMPs). So far, 13 distinct mammalian TLRs have been identified 10 of which are functional in humans (TLR1-10), and 11 in mice (TLR1-7, TLR9, TLR11-13).<sup>[4]</sup> TLRs are located on the cell surface and endosome. TLR1, 2, 4, 5, 6, 10 and 11 are located on the cell surface, whereas, TLR3, 7, 8 and 9 are located in the endosome.

TLRs have a variable number of ligand-sensing, leucine-rich repeats (LRR) at their N-terminal ends and a cytoplasmic Toll/IL-1 R (TIR) domain. The TIR domain mediates interactions between TLRs and adaptor proteins involved in signaling including MyD88 (Myeloid differentiation primary response gene 88), TRIF

(TIR-domain-containing adapter-inducing interferon- $\beta$ ), TRAM (TRIF-related adaptor molecule), etc. Signaling pathways promote the expression of pro-inflammatory cytokines and chemokines. As a result, immune cells are recruited to the infection site and the pathogenic microbes and infected cells are eliminated. Although TLRs provide protection against a wide variety of pathogens, inappropriate or unregulated activation of TLR signaling can lead to chronic inflammatory and autoimmune disorders.

In the past few decades, numerous studies have shown the significance of TLR mechanism in periodontal disease. Periodontitis can result from a number of different perturbations of host homeostasis, whether due to a shift in microbial composition or shift in host immune mediator production. It seems that a shift in dental plaque numbers and composition plays a critical role in initiating destructive innate immune inflammatory responses, which results in disease. For example study by Yoshioka et al [5] has shown that both low and high plaque samples activate both TLR2 and TLR4. Also, plaques obtained from individuals with higher plaque scores elicit significantly more TLR4 activation.

Apart from recognition of exogenous/foreign PAMPs, TLRs can sense endogenous molecular patterns. A large number of viruses have been shown to trigger innate immunity via TLRs. Nucleic-acid sensing plays an important role in recognizing viral genomes.[6] Members of the MyD88 families are providing emerging opportunities that modify innate immune responses in ways which benefit the host. The increasing role of bacterial – viral co infections in periodontal pathogenesis will definitely throw light into the MyD88 dependent and independent mechanisms involved in the process.

### 2.1.2 Retinoic Acid-Inducible Gene 1-Like Receptors (RLRS)

Retinoic acid-inducible gene 1-like receptors (RLRs) are a novel family of pattern recognition receptors that include retinoic acid-inducible gene 1 (RIG-1), melanoma differentiation-associated gene 5 (MDA-5) etc.[7] RLRs are cytosolic sensors for nucleic acids. Unlike Toll-like receptors, which sense “exogenous” pathogen-associated molecular patterns, RLRs detect “cytoplasmic” viral RNA. RIG-1 and MDA5 can sense RNA species. More recently the recognition of cytosolic DNA has been under the spotlight.[3] The first identified cytosolic DNA sensor is DNA-dependent activator of IFN-regulatory factors (DAI). So, RLRs like RIG-1,MDA5 sense RNA species, while DAI recognize DNAs.

Apart from pathogenic bacteria, viruses also have shown to impart their role in the pathogenesis of periodontitis. Viruses from the Herpesviridae family have been implicated in the pathogenesis of periodontal disease. Botero et al [8] have observed that human cytomegalo virus-HCMV detection in periodontal pockets was associated with higher levels of periodontopathic bacteria and increased probing depth and attachment loss at sampled sites. HCMV/bacteria coinfection may be an important factor in periodontal destruction. RLR like DAI which recognize viral DNA may be of help to unveiling the mechanisms of this pathogenesis. But, research is needed to determine the extent to which herpes viral–bacterial co-infections in periodontitis represent pathogenetically significant interactions or merely an independent etiologic importance of each of the infectious agents.

### 2.1.3 Nod-Like Receptors (NLRs)

NOD-Like Receptors (NLRs) constitute a family of intracellular pattern recognition receptors (PRRs), which contains more than 20 members in mammals. While responses to extracellular PAMPs are mediated by membrane bound receptors such as TLRs, many NLRs are specialized for detection of PAMPs that had reached the cytosol or subcellular organelles.

NLRs contain 3 domains – central NACHT (NOD or NBD – nucleotide-binding domain) domain, which is common to all NLRs, N terminal domain containing caspase recruitment domain (CARD), pyrin domain (PYD), Acidic transactivating domain or Baculovirus inhibitor repeats (BIRs). Based on the type of N terminal domain, NLRs have been named as NLRA (A for acidic transactivating domain), NLRB(B for BIRs), NLRC(C for CARD), and NLRP(P for PYD).

NLRs include NOD-1, NOD-2, NLRPs,NLRCs etc. NOD1 protein contains a caspase recruitment domain (CARD). NOD1 is an intracellular pattern recognition receptor and is a close relative of NOD2. Recently, Krishnaswamy et al [9] reviewed the varied roles of NOD-like receptors (NLRs), in immune responses and propose a new model in which adaptive immunity requires coordinated PRR activation within the dendritic cell.

The primary role of NLRs is to recognize “cytoplasmic” pathogen-associated molecular patterns (PAMPs) and/or “endogenous” danger signal released by the host, inducing immune responses.[10] A major inflammatory pathway of this PRR is the activation of the “inflammasomes”. [11] The term “inflammasome” collectively refers to oligomeric molecular platforms or assemblies that recruit and activate caspase-1. Once activated, Caspase-1 proteolytically cleaves pro-IL1 $\beta$ [12] and pro-IL-18[13] to IL-1 $\beta$  and IL-18, which is critical for triggering pro-inflammatory and anti-microbial responses. These assemblies are formed in the cytoplasm of innate immune cells like macrophages and dendritic cells when these cells are exposed to danger signals.

To date, four bonafide inflammasomes named by the PRR that regulates their activity have been identified: the NLRP1, NLRP3, NLRC4(all 3 belonging to the Nod-like receptor - NLR family) and AIM2 inflammasomes.<sup>[14]</sup> Absent in melanoma 2 (AIM2) is a newly discovered PRR. AIM2 is referred to as the DNA inflammasome for its ability to detect foreign double stranded DNAs.

The current widely accepted model of inflammasome assembly proposes that NLRs sense directly or indirectly the presence of pathogens. Inflammasomes are responsible for secretion of inflammatory cytokines IL-1 $\beta$ , IL-18, as well as the promotion of cell death. The IL-1 $\beta$  processing inflammasome has recently been identified as a target for pathogenic evasion of the inflammatory response by a number of bacteria and viruses. Studies in the recent past have drawn attention to the interaction between *Porphyromonas gingivalis* and NLRs. We know *Porphyromonas gingivalis* (Pg) has low immunogenicity and synergizes with other periodontal pathogens, including *Fusobacterium nucleatum* (Fn). It has been shown that Pg failed to activate an inflammasome on its own, but induced synthesis of pro-IL-1 $\beta$  through NLRP3-dependent cleavage and secretion of IL-1 $\beta$ .<sup>[15]</sup> In contrast, Taxman et al<sup>[16]</sup> showed that Pg selectively represses the activation of the IL-1 $\beta$  processing inflammasome by Fn through a novel mechanism involving modulation of endocytosis. This Inflammasome related selective suppression – repression mechanism of Pg may need to be observed further to find out missing mechanistic links of that may explain the pathogenicity of periodontitis and other chronic diseases.

Also, Bostanci et al<sup>[17]</sup> in 2009 observed that NALP3 and NLRP2 are expressed at significantly higher levels in the three forms of inflammatory periodontal disease compared to health. A positive correlation was revealed between NALP3 and IL-1 $\beta$  or IL-18 expression levels in these tissues. Pg deregulates the NALP3 inflammasome complex by enhancing NALP3 and down-regulating NLRP2 expression. This study reveals a role for the NALP3 inflammasome complex in inflammatory periodontal disease, and provides a mechanistic insight to the host immune responses involved in the pathogenesis of the disease by demonstrating the modulation of this cytokine-signaling pathway by bacterial challenge.

## 2.2 DAMPs

Besides the recognition of microbial PAMPs, the immune system senses other signals associated with infection. This includes “endogenous” host components released from infected or necrotic cells, which activate and amplify the immune response. Endogenous products released from dying or damaged cells are called DAMPs, i.e. *damage* - (from Latin ‘damnum’: ‘loss, hurt’) *associated molecular patterns*. This means that, immune response is induced by not only exogenous microbial infection, but also by endogenous sterile tissue damage and degeneration. That is, the sudden exposure of intracellular molecules or their derivatives outside the cell acts as a sign of a potential danger of cellular damage. This sudden release may have occurred by cellular stress, trauma-induced tissue damage or nonphysiological cell death such as necrosis.

During trauma or damage-induced responses, many endogenous molecules such as DNA, ATP, uric acid, DNA binding proteins, and reactive oxygen species (ROS) [DAMPs] are released from infected or stressed host cells. These endogenous molecules bind to specific DAMP receptors (DAMPs) resulting in subsequent release of cytokines and chemokines, including interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)- $\alpha$ . Cytokines and the transmigration of neutrophils and monocytes may result in endothelial dysfunction, causing vasodilatation and increased capillary permeability. Based on the common recognition system and the similar inflammatory response, some authors use the term “alarmin” (Italian ‘all’arme’: ‘to arms’) to categorize endogenous DAMPs as separate from ‘nonself’ pathogen-derived molecules.<sup>[18]</sup>

Well-known alarmins include heat shock proteins (Hsp), hyaluronan, uric acid (UA, monosodium urate), galectins, thioredoxin, adenosine, high mobility group protein 1 (HMGB1), interleukin-1 $\alpha$  (IL-1 $\alpha$ ), and interleukin-33 (IL-33),<sup>[19]</sup> S100 proteins. HMGB1, IL-1 $\alpha$ , and IL-33 exert dual functions as intracellular transcription factors and as extracellular inflammatory mediators. S-100 protein is a family of low molecular weight protein found in vertebrates characterized by two calcium binding sites. The name is derived from the fact that the protein is 100% Soluble in ammonium sulfate at neutral pH. S100 is normally present in cells derived from the neural crest (Schwann cells, melanocytes and glial cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, dendritic cells, and keratinocytes.

## III. Alarmins as Biomarkers of Diagnosis and Prognosis

Similar to inflammatory biomarkers such as CRP, erythrocyte sedimentation rate (ESR), and IL-6, certain alarmins have been identified as biomarkers of inflammation. A member of the HMGB family, HMGN1, has recently been identified as a novel alarmin that is critical for lipopolysaccharide-induced immune responses.<sup>[20]</sup> The levels of the S100 proteins has been found to correlate with disease activity in a number of inflammatory conditions.<sup>[21,22]</sup> S100 proteins show important advantages over traditional clinical or laboratory markers for specific indications, probably due to their local expression and release in direct response to tissue damage. S100A8 and S100A9, the most abundant cytoplasmic proteins of neutrophils and monocytes, are also

mediators of inflammation.<sup>[23]</sup> S100A8/S100A9 complexes are released during activation of phagocytes and mediate their effects via TLR4, leading to the production of TNF- $\alpha$  and other cytokines.<sup>[24]</sup> S100A8/S100A9 levels were elevated on exposure to minimal amounts of LPS. Blockade of S100A8 and S100A9 suppressed LPS-induced proinflammatory activities.<sup>[25]</sup> Levels were also increased in septic patients and inversely correlated with survival.<sup>[26]</sup> Serum levels of S100A8/S100A9 were found to correlate better with disease activity and joint destruction in various inflammatory arthritides than classical markers of inflammation.<sup>[27]</sup>

Much research has been done to assess the levels of inflammatory marker CRP, ESR, IL-6 etc in chronic periodontitis. Since periodontitis shares common immunomodulatory profile with Rheumatoid Arthritis, the role of alarmins in chronic inflammatory condition like periodontitis need to be evaluated.

#### **IV. Alarmins in Regenerative Therapy**

The effects of alarmins, whether beneficial or detrimental, appear to depend on timing of release, dose, and context. Excessive and chronic presence of alarmins and unremitting alarmin-induced events exacerbate injury, but when expressed in a transient and self-limited manner upon injury and acute inflammation, they mediate repair. Further researches are the need of the hour to check the quantitative and qualitative effects of alarmins on the host.

Thus far, research on the use of alarmins in regenerative therapy is limited to preclinical studies. Exogenous application of alarmins has shown promise in cutaneous wounds. Skin wound repair is problematic in diabetes mellitus due to a dysregulated inflammatory response compounded by an increased microbial load, excessive protease activity, and vascular compromise.<sup>[28]</sup> The antimicrobial alarmins are particularly attractive for cutaneous wound healing due to their additional antimicrobial activities. S100A8 and S100A9 also appear to promote skin wound healing.<sup>[29]</sup> The role of alarmins as possible regenerative material for periodontal wounds need to be assessed.

#### **V. Challenges And Future Directions**

The therapeutic potential for immunomodulation by targeting alarmins and their signaling pathways appears promising and needs to be tested in clinical trials. However, there are several key issues that remain to be addressed. This is likely to be achieved by a better understanding of how the microenvironment and dosage contribute to the net effects of alarmins.

#### **VI. Summary**

To summarise the terminologies, during an infection in vivo, the innate immunity immediately responds once encountered by PAMPs or DAMPs. The detection of endogenous and exogenous alarm signals is possibly accomplished by the same early recognition systems (PRRs)-TLRs, RLRs, NLRs. PAMPs are exogenous pathogen related detected through TLRs, cytosolic viral RNAs and DNA s through RLRs, while endogenous host derived damage molecules DAMPs (sometimes termed as alarmins), and inflammasome activation is related to NLR pathway mainly.

#### **VII. Concluding Remarks**

Despite substantial progress in understanding the host immunology many gaps remain in our understanding of this sophisticated danger and infection detection system. The host innate immune system, is able to respond to “patterns of pathogenesis”, i.e. signals related to the strategies that live pathogens use to invade host cells, replicate intracellularly, and spread through tissues. Regardless of the lessons that may be learned in the future, the discovery of the PRRs and elucidation of their function have already contributed critical information for understanding how an effective innate immune response is established. Further understanding of apparently conflicting roles of PRRs in inflammation and repair is beginning to yield novel approaches for translation to the clinical arena not only in the medical field but also in Periodontics .

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