Triggering receptor expressed on myeloid cells in the pathogenesis of periodontitis: potential novel treatment strategies

Courtney P. Rudick[a], **Takanari Miyamoto**[b], **Melissa S. Lang**[b], and **Devendra K. Agrawal**[a]

[a]Department of Clinical & Translational Science, Creighton University School of Medicine, Omaha, NE, USA

[b]Department of Periodontology, Creighton University School of Medicine, Omaha, NE, USA

Abstract

Introduction—Periodontal diseases are polymicrobial inflammatory disorders of the tissue, ligament, and bone structures supporting teeth. Periodontitis (inflammation with corresponding loss of attachment) affects 40–50% of adults. Recently, members of the Triggering Receptor on Myeloid Cell (TREM) family have been studied to determine their relationship to these diseases.

Areas covered—TREM-1 is a receptor expressed on the surface of PMNs, monocytes, macrophages, dendritic cells, vascular smooth muscle cells, and keratinocytes upregulated in the presence of periodontal inflammation. TREM-1 expression can be upregulated by oral bacterium *Porphyromonas gingivalis* that can be abrogated by a sub-antimicrobial dose of doxycycline. When cleaved from the cell surface, a soluble form of TREM-1 (sTREM-1) can be used as a biomarker of inflammation and might also provide a link between oral and systemic inflammation. While less understood, TREM-2 has a role in osteoclastogenesis which could contribute to the alveolar bone destruction seen in more advanced periodontitis.

Expert Commentary—Additional studies to simulate biofilm microenvironment in TREM research are warranted. Longitudinal studies determining TREM-1, sTREM-1, and TREM-2 levels in tissues over time and progression of periodontal diseases would provide valuable information in the role of TREM receptors as indicators of or contributors to the disease process.

Keywords

Gingivitis; Inflammation; Periodontal Diseases; Periodontitis; *Porphyromonas gingivalis*; sTREM-1; TREM-1; TREM-2

[*]*Corresponding author.* Devendra K. Agrawal, Ph.D. (Biochem), Ph.D. (Med. Sciences), MBA, MS (ITM), FAAAAI, FAHA, FAPS, FIACS, Professor and Chairman, Department of Clinical & Translational Science, The Peekie Nash Carpenter Endowed Chair in Medicine, Senior Associate Dean for Clinical & Translational Research, Creighton University School of Medicine, CRISS II Room 510, 2500 California Plaza, Omaha, NE, 68178, USA, Tel: (402) 280-2938; Fax: (402) 280-1421, dkagr@creighton.edu.
ORCID
Devendra K Agrawal ID http://orcid.org/0000-0001-5445-0013

1. Introduction

Periodontal diseases include gingivitis and periodontitis that are inflammatory diseases affecting the supporting structures of the teeth which can lead to connective tissue destruction, alveolar bone loss and edentulism [1]. Gingivitis is a reversible inflammation caused by plaque which leads to redness, swelling, and bleeding of the gingival tissues without loss of attachment (LOA) [1]. Periodontitis is the irreversible progression of inflammation characterized by the breakdown of connective tissue leading to the apical migration of the junctional epithelium and accompanied by mild, moderate, or severe loss of alveolar bone [1]. The American Academy of Periodontology estimates 5–15% of Americans are affected by severe periodontitis and 30% of Americans have greater than 4mm probing depths on at least three teeth indicating the presence of periodontal disease [2]. The World Health Organization puts the estimate slightly higher with 40–50% of adults having greater than 4mm probing depths, and the percentage increases drastically with age [3].

Periodontal infections have been associated with systemic inflammatory diseases [4]. Using polymerase chain reaction (PCR), oral bacteria *Streptococcus mutans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola* and *Aggregatibacter actinomycetemcomitans* have all been detected on cardiac valve samples [5,6]. Antibodies to oral gram-negative (gm−) bacteria have been detected in the synovial fluid of patients with rheumatoid arthritis (RA) and it has been found that patients with RA are more likely to develop periodontitis despite age and smoking history; even further, patients with periodontal infections are less responsive to RA treatments [4]. Non-surgical periodontal therapy (NSPT) has shown a beneficial effect on inflammation of the vascular wall attributed to periodontitis by lowering plasma interleukin (IL)-6, improving the lipid profile, and improving elasticity of the aortic artery [7]. Though there has not been definitive research establishing the link between periodontal and systemic inflammation, there has been research showing NSPT can reduce bacterial challenges, resolve inflammation, and restore health [4,8,9].

Periodontal diseases are caused by bacteria commonly arranged in biofilms [1]. In general, the oral cavity can host over 6 billion bacteria from over 700 species (500 of which are able to arrange in biofilms), with up to 200 species present in an individual mouth at a given point in time [10,11]. Oral bacteria are a mix of gram-positive (gm+) and gm−, aerobic, anaerobic, and facultative anaerobic bacteria; as well as fungi, viruses, mycoplasms, and protozoa [10,11]. The oral cavity is an ideal environment for bacterial colonization because it is constantly moist and dark, has a neutral pH, and maintains a consistent temperature of 34–36°C [10]. Gram-positive species, including *S. mutans, Streptococcus gordonii,* and *Streptococcus oralis*, are thought to be early colonizers of the oral cavity which can live in harmony with the host immune system, possibly even promoting periodontal health [12,13]. The destruction typically seen in periodontitis is associated with a switch from gm+, facultative, fermentative microorganisms to gm− anaerobes [14]. Specifically, three "red complex" bacteria (*P. gingivalis, Tanerella forsythia,* and *T. denticola*) and *A. actinomycetemcomitans* are associated with periodontal tissue destruction [15,16]. No single species is responsible for the development of periodontal diseases; rather, the interaction of

bacteria and inflammatory responses of the host while attempting to protect tissues from bacterial challenges are the causative factors [10, 17].

Biofilms are a conglomeration of microorganisms in their extracellular polymer matrix which bind together and attach to host tissues; because of the biofilm structure, these microorganisms are highly resistant to antimicrobial agents [13]. Within biofilms, bacteria can live and reproduce in protected environments [13]. Bacteria in biofilms associate through physical contact, metabolic exchange, and signal-mediated communications; all of which can determine the structural characteristics and virulence of a specific biofilm [15]. Bacterial associations in a protected biofilm allows for the growth of species which would not be able to survive independently [13]. Another virulence factor of biofilms is the ability of the subgingival bacteria to alter host protein expression [18]. Bacteria in biofilms are able to downregulate host proteins associated with tissue integrity and phagocytic pathway signaling while increasing proteins responsible for inflammation (i.e. specifically through altering gingival fibroblast transcription to promote an amplified innate immune response) and apoptosis thereby reducing the host's ability to effectively clear bacteria [18,19]. Microorganisms in biofilm are also capable of downregulating protein expression for desmosomal junctions in epithelium allowing for an increase in invading pathogens [20].

Oral microbes express pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), which are recognized by toll-like receptors (TLRs), nod-like receptors (NLRs), and other pattern recognition receptors on innate immune responders, opsonins, and host cells. These molecules, in turn, activate neutrophils and other leukocytes and produce proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1β, and IL-6 [13,21]. Neutrophils are found extensively in the junctional epithelium and migrate to the sulcus and gingival crevicular fluid (GCF) [13]. Once activated, neutrophils engage in phagocytosis, degranulation, generation of reactive oxygen species (ROS), and formation of neutrophil extracellular traps (NETs) in order to control and eliminate invading species. NETs allow neutrophils to immobilize vast amounts of microorganisms which are too numerous for individual phagocytosis [13]. However, neutrophil enzymes designed to protect the host can degrade collagen and other host proteins adjacent to the biofilm, inadvertently contributing to periodontal tissue destruction [13]. TLRs (especially TLR-2, TLR-4 and TLR-5) can activate neutrophil adhesion to form a wall against biofilm bacteria [22]. As mentioned above, biofilms are protected against neutrophil attack leading to a phenomenon of "frustrated phagocytosis" which results in the recruitment of excessive amounts, or hyperactive neutrophils [22]. As neutrophil activity increases, so does collateral tissue damage to periodontal structures [21,22].

Polymorphonuclear leukocytes (PMNs) are the most abundant leukocytes (50–70% of all white blood cells) and act as primary defense mechanism for host cells [9,21]. As a result of proinflammatory signals, PMNs attach firmly to endothelial lining of blood vessels within the gingival lamina propria [21]. Every minute, 30,000 PMNs travel through periodontal tissues mediated by signals from IL-8 and Intercellular Adhesion Molecule-1 (ICAM-1); while typically cleared through apoptosis, increased PMN survival is a leading contributor to the chronic inflammatory state seen in periodontitis [9].

Triggering Receptor Expressed on Myeloid Cells-1 (TREM-1) is a receptor expressed on the surface of PMNs, monocytes, macrophages,, dendritic cells, vascular smooth muscle cells, (and some keratinocytes) and is upregulated in the presence of inflammation [21,23,24]. TREM-1 is located on human chromosome 6p21 and belongs to the immunoglobulin super family along with TREM-2, TREM-3 (a pseudogene in humans, but a functional gene in mice), and homologous genes TREM-like transcript 1 (TLT-1) and TLT-2 [25]. TREM proteins are highly conserved in evolution and are also present in chickens, pigs, and cows. Murine and human TREM genes cluster closely, as do Bovine and pig TREM gene expression [25]. TREM-1 in humans activates downstream signaling pathways through adaptor protein DNAX-activating protein of 12 kDa (DAP12) as an amplifier of the immune response [21,25].

2. Triggering Receptor Expressed on Myeloid Cells (TREM)-1

Triggering Receptor Expressed on Myeloid Cells-1 is upregulated during bacterial and fungal infection as demonstrated by increased expression on the surface of activated monocytes (primarily), neutrophils, and some keratinocytes [21,26]. TREM-1 is activated by a currently unknown natural ligand, but may also be activated or upregulated by several other molecules (see Table 1). A synthetic control peptide (LR12) can be used to inhibit TREM-1 activation [25]. LR12 is designed to mediate TREM-1 receptor dimerization through interaction with the TREM-1 complementary determining region 3 and the F-Beta strand of the extracellular domain [25]. Other potential inhibitors of TREM-1 can be found in Table 2. TLR-induced signals (especially TLR-2) are amplified by activated TREM-I leading specifically to an increase in cytokine (IL-1α, TNF-α, Granulocyte-macrophage colony-stimulating factor (GM-CSF)) and chemokine (IL-8, Monocyte chemotactic protein 1 (MCP-1), MCP-3, Macrophage Inflammatory Proteins (MIP-1) production [27]. Blocking TREM-1 decreases Th1 signaling through IL-1β, Interferon (IFN)-γ, and IL-6 and increases Th2 production of IL-4, IL-5, and IL-10 [8, 28, 29]. Toll-like Receptors (TLRs) are germ line encoded receptors of the innate immune response responsible for distinguishing between microorganisms and responding appropriately to specific microbes [30]. Synergism between TREM-1 and TLRs leads to an increased production of proinflammatory cytokines TNF-α and IL-1β, and inhibits the anti-inflammatory molecule IL-10 [31]. TREM-1 acts synergistically with TLR-2 to induce cytokine production, while TLR-2 can also upregulate TREM-1 via the MyD88-dependent pathway [32]. TREM-1 is able to positively modulate TLR-4 through regulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which in turn modifies MyD88 and cluster of differentiation 14 (CD14) expressions [32]. TREM-1 can activate the NF-κB pathway leading to increased production of TNF-α, IL-2 and IL-1β [31,32].

DAP12 is a transmembrane protein with an immunoreceptor tyrosine-based activation motif (ITAM) signaling unit [33]. TREM-1 consists of an extracellular domain, a transmembrane region, and a short cytoplasmic domain which lacks any signaling motifs [32]. TREM-1 signaling requires the formation of an intracellular complex with DAP12. Upon recognition of bacterial challenge by TLRs and PAMPs, TREM-1/DAP12 interactions mediate downstream signaling to increase proinflammatory cytokine production [27]. Research conducted by Bostanci et al. [8] revealed *P. gingivalis* can disrupt the TREM-1/DAP12

signaling pathway. Through this mechanism, *P. gingivalis* was determined to have engagement with TREM-1 and may act to regulate systemic inflammation [8].

When mononuclear phagocytes are combined with agonistic TREM-1 antibody, followed by pattern recognition receptors (e.g. TLRs and NLRs) the result is a synergistically significant increase in cytokine and chemokine secretion when compared to either component alone [31].

On an mRNA level, TREM-1 is expressed in significantly higher levels in tissue samples with chronic periodontitis (CP) and generalized aggressive periodontitis (GAP) compared to healthy control samples (3-fold and 13-fold respectively), but that there was no significant difference between CP and GAP [34]. There were significant positive correlations between TREM-1 expression and all three red complex bacteria (not including *A. actinomycetemcomitans*) as well as presence of bleeding on probing (BOP) and levels of IL-1β production [34].

Using immunohistochemical staining, in gingival epithelium TREM-1 was expressed in the cytomembrane, cytoplasm, and extracellular matrix (predominantly the spinous and basal layers); however, there was only weak expression of TREM-1 in subepithelial connective tissues [35]. Overall, TREM-1 was expressed in 86.7% of healthy biopsies and 100% of biopsies from inflamed tissues [35]. TREM-1 expression showed a moderate, but statistically significant, positive correlation with BOP, periodontal probing depth (PPD), and clinical attachment levels signifying its role in inflammation [35].

3. Soluble Triggering Receptor Expressed on Myeloid Cells (sTREM)-1

A soluble form of triggering receptor expressed on myeloid cells-1 (sTREM-1) has also recently been studied in relation to inflammatory diseases such as sepsis, rheumatoid arthritis, periodontal diseases, and acts as an important biomarker in sepsis as well as bacterial and fungal pneumonia [16]. sTREM-1 is most likely produced through the proteolytic cleavage of membrane bound TREM-1 by matrix metalloproteinases [17]. Because sTREM-1 has the same extracellular domain as TREM-1, sTREM-1 might competitively bind the natural ligand of TREM-1 in order to downregulate TREM-1 signaling pathways [36].

In GCF analysis using sandwich ELISA techniques, sTREM-1 levels are significantly increased in pathologic sites of periodontitis when compared to healthy samples or control sites of healthy gingiva in patients with periodontal disease [21]. There was a significant increase of sTREM-1 from pockets with PPD 0–3mm to PPD 5–7mm, but not again for pockets with a PPD of 8+mm [21]. sTREM-1 levels varied between participants and between sites in the same participant and were higher in mobile teeth; smoking affected sTREM-1 levels, but the impact did not reach significance [21]. Bisson et al. [21] posit increased sTREM-1 levels could be due to positive feedback from downstream inflammatory factors reflecting the host's inability to clear infection. The lack of measurable sTREM-1 elevation as periodontitis advances clinically could be due in part to bacterial proteolysis of sTREM-1 or the progression of the disease from an acute to chronic state.

In another study evaluating GCF samples of 62 participants (20 controls, 22 with CP, and 20 with GAP each with at least 20 teeth) [16]. sTREM-1 concentrations were measured by ELISA and site specific subgingival levels of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, and *T. denticola* were also measured. All bacteria counts were higher in CP and GAP compared to healthy sites, but *A. actinomycetemcomitans* levels were too low from any site to be analyzed meaningfully [16]. Comparable to other studies, there was no significant difference in bacterial load from CP to GAP samples [16]. sTREM-1 was detected in all but two samples, both from healthy sites; however, there was no significant difference between concentrations for any group [16]. The research team posits these results show a misleading decrease in sTREM-1 concentrations owing to the fact that GCF is not produced without inflammation, so healthy samples have a much lower amount of fluid than inflamed samples [16]. When comparing bacterial load to sTREM-1 levels, there was significant positive correlation, with *P. gingivalis* showing the strongest correlation to sTREM-1 levels in GCF [16].

In GCF samples from elderly patients, there was sTREM-1 expression in all samples with no significant differences between healthy participants, or participants with gingivitis or periodontitis [37]. The researchers posit this elevation is due to a dysregulated immune response in elderly populations due to compromised function of monocytes and macrophages (e.g. reduced chemotaxis, phagocytosis, production of reactive oxygen, and chemokine response) [37]. The authors note limitations in the study, specifically the study is cross-sectional in nature which does not allow for continuous monitoring of sTREM-1 levels over time [37].

Recently, high mobility group box protein B1 (HMGB-1) has been studied in relation to sTREM-1 and periodontal diseases. HMGB-1 is a Damage-associated molecular pattern (DAMP) secreted by inflammatory cells and interacts with TLR's (primarily TLR-2, TLR-4, and TLR-9) to enhance proinflammatory cytokine expression [17]. One study showed a significant positive correlation between HMGB-1 and sTREM-1 levels in patients with chronic periodontitis [17]. HMGB-1 is known to play a role in osteoclastogenesis, and combination with sTREM-1 in the presence of periodontitis could lead to increased tissue destruction [17]. The authors posit HMGB-1 and sTREM-1 levels can be used as diagnostic markers for the degree of tissue destruction seen in periodontitis [17].

In a comparison via ELISA of serum and salivary levels of sTREM-1 in 59 individuals (18 controls, 20 with CP, and 21 with GAP), there was a 3.3-fold increase in the CP group and a 5.6-fold increase in the GAP group. The difference between sTREM-1 levels in the CP and GAP groups did not reach significance [26]. Serum levels of sTREM-1 showed a 1.75- and 2-fold increase for CP and GAP, respectively, again, the research showed no significant difference between CP and GAP [26]. There was a positive correlation between salivary and serum sTREM-1 levels (with salivary levels being two times higher than serum) which could provide a link between the inflammation seen in periodontal and systemic diseases [26]. There was a positive correlation between salivary sTREM-1 levels and IL-1β, in keeping with findings from previous studies [8] showing TREM-1 also increases IL-1β [26]. More challenging to the group was determining how sTREM-1 appears in serum, offering two suggestions: sTREM-1 expression in serum could be due to circulating leukocytes present in

systemic infection exposed to periodontal pathogens, or due to sTREM-1 produced locally at the site of periodontal infection somehow being leaked into the bloodstream; more research needs to be conducted in this area [26]. The authors acknowledge small sample size and cross-sectional nature of the study as two limitations. As such, the results can be used to confer association, but no knowledge could be gained as to the mechanism of action or predictive value of salivary/serum sTREM-1 levels [26]. More research should be conducted to determine the value of sTREM-1 in GCF as a predictive biomarker for periodontal disease, or as an indicator of oral/systemic inflammation interactions [26].

4. Regulation of TREM-1 by *Porphyromonas gingivalis*

Porphyromonas gingivalis is a gm−, obligate anaerobe (also intracellular facultative), asaccharolytic rod bacterium. Part of the red complex, *P. gingivalis* plays several roles in determining the virulence of biofilms and corresponding level of host inflammatory response [10,21,22,38,39]. Fimbriae, lipopolysaccharide (LPS), and cell surface cysteine proteinases (gingipains) all contribute to the virulent capabilities of *P. gingivalis* [15]. *P. gingivalis* is able to survive and replicate within host cells, the bacterium then returns to the extracellular space where it escalates the host immune response before retreating inside the host's cells [21]. The ability of *P. gingivalis* to migrate within and without host cells could play a major role in the remission and refractory nature of chronic periodontitis [40]. *P. gingivalis* is a secondary colonizer (often adhering to *S. gordonii* and *P. intermedia*) and is considered a "keystone species" in the development of periodontitis [10]. One beneficial reason for *P. gingivalis* to exacerbate inflammation is to encourage blood flow to the area, providing an iron rich source of nutrition while also upregulating host genes associated with acquisition of iron from hemin [10, 41]. *P. gingivalis* is able to diminish the host's immune response by reducing the amount of CD14, thereby reducing macrophage responsiveness [10]. The bacterium secretes SerB phosphatase, a potent and specific inhibitor of NF-κB activation which in turn reduces IL-8 production [22]. *P. gingivalis* can suppress superoxide anions (O_2^-) preventing the bactericidal activity associated with neutrophil oxidative bursts, and can also inactivate: cathespin G, elastase, bacterial-permeability increasing factor, and defensins allowing the bacterium to evade host defenses [22].

P. gingivalis has two gingipains, Arginine specific (RgpA) and Lysine specific (Kgp), both of which are anaerobic and utilize free amino acids as a source of carbon and nitrogen. [15]. *P. gingivalis* relies on its gingipains to mediate iron uptake from hemoglobin, heme proteins, and ferritin along with a host of other functions (see Figure 1) [15]. Arg- and Lys-gingipains also help evade host antimicrobial response by degrading antibacterial peptides (specifically neutrophil derived alpha-defensins, complement factors C3 and C4, T-cell receptors CD4 and CD8) and by disrupting cross talk between C5a receptors and TLR signaling [15, 42]. Gingipains are also able to regulate inflammatory mediators including: proinflammatory cytokines IL-1α/β, IL-18, receptor activator of nuclear factor kappa-B ligand (RANKL) which impacts bone remodeling, proinflammatory molecule TNF-α converting enzyme (TACE), and a receptor which increases inflammation when activated: protease activated receptor-2, and sTREM-1 [15]. The Arg-gingipain is able to shed TREM-1 from the cell surface of neutrophils causing an increase in sTREM-1, thereby amplifying the host's immune response [22]. The Lys-gingipain is able to degrade sTREM-1 in order to obstruct

neutrophils from maintaining the host's immune response including phagocytosis [22]. The dual regulation of the immune response allows *P. gingivalis* to evade host immune cells when inflammation is high, but encourage inflammation if nutrient sources are low [22]. Research conducted by Bao et al. (2012) demonstrated other effects of the Arg- and Lys-gingipains [15]. Using a ten-species constructed biofilm, structure and arrangement were evaluated by confocal laser scanning microscopy and bacterial species numbers determined by fluorescence in situ hybridization (FISH) or immunofluorescence. *T. forsythia* was determined to be dependent on the Lys-gingipain for growth, and *T. denticola* aggregates in the presence of the Arg-gingipain - where otherwise it grows in loose threadlike structures [15].

Quantitative Reverse Transcription-PCR (QRT-PCR) results showed *P. gingivalis* amplified TREM-1 expression after four hours, followed by an increase in sTREM-1 over 18 hours as determined by enzyme-linked immunosorbent assay (ELISA) - at which point PMNs had lower surface staining of TREM-1 as identified by flow cytometry and confocal laser scanning microscopy [43]. Engaging TREM-1 increased IL-8 production by 30% and when combined with *P. gingivalis* increased IL-8 production 35%; in contrast, antagonizing TREM-1 decreased IL-8 production by 30% alone or 25% when combined with *P. gingivalis* [43]. The Arg-gingipain sheds sTREM-1 from the surface of PMNs while the Lys-gingipain is able degrade sTREM-1 [43]. Therefore, *P. gingivalis* is able to stimulate TREM-1 expression on neutrophils and mononuclear cells, and can promote the shift from membrane bound TREM-1 to sTREM-1 accompanied by an increase in cytokine production [8,43]. The effect of *P. gingivalis* on TREM-1 can be abrogated by the administration of subantimicrobial levels of doxycycline between 2–10μg/mL^{-1} [27]. Doxycycline is a member of the tetracycline family of antibiotics, but at subantimicrobial levels does not alter antibiotic susceptibility of oral or intestinal flora. Inhibition of TREM-1 production by this dosage of doxycycline may contribute to the molecular mechanism which reduces excessive inflammation in periodontal infections caused by *P. gingivalis* [27].

5. TREM-2 and Inflammation

Currently, there is less thorough understanding regarding the role of TREM-2 in inflammation and infection compared to other members of the TREM family. TREM-2 is thought to be a negative regulator of the inflammatory response; however, there has been very little study in regards to TREM-2 and periodontal diseases [31]. Chen, Wang, & Zhao [35] conducted the first experiments with TREM-2 and periodontal diseases. There was increased expression of TREM-2 in diseased gingival tissues compared to healthy controls [35]. There was no TREM-2 expression in healthy gingival epithelium samples but found 50% of inflamed epithelial samples showed TREM-2 expression. Similarly, in periodontal connective tissue samples only 13.3% showed expression of TREM-2; however, 50% of the inflamed connective tissue samples showed TREM-2 expression [35]. TREM-2 promotes dendritic cell maturation and survival. TREM-2 is also able to act as a phagocytic receptor by recognizing and binding several species of fungi and bacteria thereby improving a host's immune response through improved microbial clearance [35]. These findings could conflict with a 2009 study that showed *P. gingivalis* was responsible for the down-regulation of

TREM-2 expression; however, more research needs to be conducted before it will be possible to establish a specific relationship [44].

There are multiple osteoimmunological similarities between rheumatoid arthritis and periodontitis [45]. While there has not yet been extensive research regarding TREM-2 and periodontal diseases, there has been research concerning the relationship between TREM-2 and RA. Significantly higher levels of TREM-2, DAP12, osteoclast-associated receptor (OSCAR), and FcRγ were found in the synovial fluid of individuals with active RA compared to samples from participants with osteoarthritis or healthy tissue samples [46]. TREM-2 was expressed on mononuclear cells in lymphoid aggregates as well as on fibroblasts [46]. In relation to RA the ITAM pathway is responsible for effecter immune cell proliferation, differentiation, and survival, as well as provision of costimulatory signals for osteoclasts [47]. There was an association between ITAM adaptor proteins DAP12 and FcRγ with TREM-2 and OSCAR (respectively). DAP12 and TREM-2 are necessary for osteoclast differentiation via Syk tyrosine kinase phosphorylation. This finding is in keeping with research showing mutations in TREM-2 or DAP12 lead to inefficient or delayed differentiation of osteoclasts, and osteoclasts from TREM-2 deficient individuals failed to differentiate into effective bone resorbing cells [47]. Considering the important role of TREM-2 in rheumatoid arthritis, and the similarities between periodontitis and RA, more research needs to be conducted on ITAM factor expression and TREM-2 in periodontal diseases.

6. Conclusion

Periodontal diseases are polymicrobial inflammatory disorders of the tissue, ligament, and bone structures supporting teeth. Periodontitis (inflammation with corresponding attachment loss) affects 40–50% of adults. Bacteria in biofilms are especially protected against host immune responses which can lead to a chronic hyperinflammatory state and significant periodontal tissue destruction. Recently, members of the TREM family have been studied to determine their relationship to the diseases. TREM-1 is a receptor expressed on the surface of PMNs, monocytes, macrophages, dendritic cells, vascular smooth muscle cells, and some keratinocytes and is upregulated in the presence of periodontal inflammation. TREM-1 expression can be upregulated by oral bacterium *P. gingivalis*, but this effect can be abrogated by a subantimicrobial dose of Doxycycline. Along with TREM-1 modulation, *P. gingivalis* has a vast array of other methods for evading host immune cells while manipulating the immune response to provide protection and nutrition. When cleaved from the cell surface, sTREM-1 can be used as a biomarker of disease as it is upregulated in the presence of oral inflammation. Positive correlations between salivary and serum levels of sTREM-1 might also provide a link between oral and systemic inflammation. While currently less understood, TREM-2 has a role in osteoclastogenesis and is upregulated during periodontitis which could contribute to the alveolar bone destruction seen in more advanced cases of periodontitis.

7. Expert Commentary

There is a large body of knowledge regarding TREM receptors in systemic inflammatory conditions; however, research is still in the initial phases of recognizing the involvement of TREM family receptors in relation to periodontal diseases [24, 48–52]. Currently most studies are in vitro assessments of bacteria and TREM family interactions, or cross-sectional studies evaluating protein expression. In order to truly understand the role of TREM-1, sTREM-1, and TREM-2 in the progression of periodontal diseases, from gingivitis to periodontitis, longitudinal studies need to be developed and implemented to evaluate protein expression over the course of the disease. Research using one or two bacteria should be modified to include the use of constructed biofilm conditions as interactions between different species of bacteria can have a large impact on host immune and inflammatory responses [34].The interplay between TREM-1 and sTREM-1 has begun to be elucidated, yet there is still little evidence of how TREM-2 interacts with the other receptors. Even though there is a growing body of knowledge for the involvement of TREM-2 in osteoclastogenesis, there is currently no published literature about the role of the protein in the alveolar bone destruction seen in periodontitis.

As for sTREM-1, there is a significant body of knowledge considering the receptor's role as a biomarker for many systemic inflammatory conditions and diseases, and there is growing evidence of sTREM-1 being upregulated in the case of periodontal inflammation [16,17]. The next step is to determine if sTREM-1 acts as a disease marker only, or if the receptor has an impact on disease progression and severity. Research needs to be done to determine if sTREM-1 levels can be used as a diagnostic or prognostic marker for periodontal diseases allowing for earlier therapeutic interventions and possible arrest of the inflammation which leads to bone destruction.

8. Five-year View

Interactions between *P. gingivalis* and TREM-1 are fascinating. The fact that a bacterium can so artfully manipulate human immune responses should be the focus of considerable research involving *P. gingivalis,* as well as other commensal and pathogenic bacteria. With greater understanding of *P. gingivalis* mechanisms, researchers will be able to find better methods of counter-manipulation to prevent bacterial evasion and disease progression.

The role of TREM-1 in systemic inflammatory conditions such as cancer, cardiovascular disorders, obesity, allergies, sepsis and pneumonia is beginning to be better understood as the body of knowledge continues to grow [24, 48–52]. As TREM-1 is increasingly studied in systemic conditions as well as in regards to periodontal diseases, scientists will be able to draw connections between oral and systemic inflammation. Connecting oral diseases with systemic conditions will allow for better treatment of patients as a whole, and more successful therapies for specific conditions.

There is still considerable research that needs to be completed to explain the specific contributions of TREM proteins in inflammatory diseases. The overarching goal of this research should be finding a way to use TREM receptors as interventional methods to

reverse or arrest progression for periodontal diseases and systemic infections. There are several studied methods of TREM-1 manipulation, all of which could provide novel treatment methodologies for periodontal disease [17,21,27,33,53]. Synthetic TREM-1 blockade could mitigate the host inflammatory response and be useful as an adjunct therapy for the treatment of periodontal disease. Hopefully, within five years, research will have shown the specific role of sTREM-1 in inflammatory conditions and diagnostic tests will be available for clinical use in dental practices to assist in patient care. In five years research will also have demonstrated the ideal form of TREM-1 modulation to provide therapeutic effects and arrest the tissue destruction common in periodontitis.

Acknowledgments

References

Reference annotations

*Of interest

**Of considerable interest

1. Darveau RP. Periodontitis: A polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010; 8(7):481–490. [PubMed: 20514045]

2. AAP Position Paper. Epidemiology of periodontal diseases. J Periodontol. 2005; 76:1406–1419. [PubMed: 16101377]

3. Petersen PE, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. Periodontol 2000. 2012; 60(1):15–39. [PubMed: 22909104]

4. Otomo-Corgel J, Pucher JJ, Rethman MP, et al. State of the science: Chronic periodontitis and systemic health. J Evid Based Dent Pract. 2012; 12(S1):20–28. [PubMed: 23040337]

5. Oliveira FAF, Forte CPF, Silva PG, et al. Molecular analysis of oral bacteria in heart valve of patients with cardiovascular disease by real-time polymerase chain reaction. Medicine. 2015; 94:e2067. [PubMed: 26632711]

6. Westling K, Vondracek M. Actinobacillus (Aggregatibacter) actinomycetemcomitans (HACEK) identified by PCR/16S rRNA sequence analysis from the heart valve in a patient with blood culture negative endocarditis. Scand J Infect Dis. 2008; 40(11–12):981–983. [PubMed: 18720255]

7. Cui D, Houxuan L, Lei L, et al. Nonsurgical periodontal treatment reduced aortic inflammation in ApoE –/– mice with periodontitis. SpringerPlus. 2016; 5:940. [PubMed: 27386384]

8*. Bostanci N, Thurnheer T, Belibasakis GN. Involvement of the TREM-1/DAP12 pathway in the innate immune responses to *Porphyromonas gingivalis*. Mol Immunol. 2011; 49:387–394. Study determines the relationship between *P. gingivalis* and the TREM-1/DAP12 communication pathway. [PubMed: 21967868]

9. Sima C, Glogauer M. Neutrophil dysfunction and host susceptibility to periodontal inflammation: Current state of knowledge. Curr Oral Health Rep. 2014; 1:95–103.

10*. How KY, Song KP, Chan KG. *Porphyromonas gingivalis*: An overview of periodontopathic pathogen below the gum line. Front Microbiol. 2016; 7:53. This is a thorough review of *P. gingivalis* and its ability to manipulate host responses to evade destruction. [PubMed: 26903954]

11. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol Oral Microbiol. 2012; 27(6):409–419. [PubMed: 23134607]

12. Murdoch C, Aziz HA, Fang HY, et al. Khat (Catha edulis) alters the phenotype and antimicrobial activity of peripheral blood mononuclear cells. J Ethnopharmacol. 2011; 138:780–787. [PubMed: 22063724]

13*. Hirschfeld J. Dynamic interactions of neutrophils and biofilms. J Oral Microbiol. 2014; 6:26102. This article provides a detailed explanation of biofilm composition and interactions on oral cavity tissues. [PubMed: 25523872]

14. Eloe-Fadrosh EA, Rasko DA. The human microbiome: From symbiosis to pathogenesis. Annu Rev Med. 2013; 64:145–163. [PubMed: 23327521]

15. Bao K, Belibasakis GN, Thurnheer T, et al. Role of *Porphyromonas gingivalis* gingipains in multi-species biofilm formation. BMC Microbiology. 2014; 14:258. [PubMed: 25270662]

16. Belibasakis GN, Ozturk VO, Emingil G, et al. Soluble triggering receptors expressed on myeloid cells 1 (sTREM-1) in gingival crevicular fluid: Association with clinical and microbiologic parameters. J Periodontol. 2014; 85(1):204–210. [PubMed: 23659423]

17. Paknejad M, Sattari M, Roozbahani Z, et al. Relationships between high-mobility group protein B1 and triggering receptor expressed on myeloid cells concentrations in gingival crevicular fluid and chronic periodontitis. Iran J Allergy Asthma Immunol. 2016; 15(5):381–385. [PubMed: 27917624]

18*. Bostanci N, Bao K, Wahlander A, et al. Secretome of gingival epithelium in response to subgingival biofilms. Mol Oral Microbiol. 2015; 30:323–335. The information on the complete secretome of gingival epithelium during bacterial challenge is provided. [PubMed: 25787257]

19. Belibasakis GN, Bao K, Bostanci N. Transcriptional profiling of human gingival fibroblasts in response to multi-species in vitro subgingival biofilms. Mol Oral Microbiol. 2014; 29:174–183. [PubMed: 24758474]

20. Belibasakis GN, Kast J, Thurnheer T, et al. The expression of gingival epithelial junctions in response to subgingival biofilms. Virulence. 2015; 6(7):704–709. [PubMed: 26305580]

21*. Bisson C, Massin F, Lefevre PA, et al. Increased gingival crevicular fluid levels of soluble triggering receptor expressed on myeloid cells (sTREM) -1 in severe periodontitis. J Clin Periodontol. 2012; 39:1141–1148. Comparative evaluation of sTREM-1 levels in GCF of patients with periodontal diseases is provided. [PubMed: 23067264]

22. Olsen I, Hajishengallis G. Major neutrophil functions subverted by *Porphyromonas gingivalis*. J Oral Microbiol. 2016; 8:30936. [PubMed: 26993626]

23. Rai V, Rao VH, Shao Z, et al. Dendritic cells expressing triggering receptor expressed on myeloid cells-1 correlate with plaque stability in symptomatic and asymptomatic patients with carotid stenosis. PLoS One. 2016; 11(5):e0154802. [PubMed: 27148736]

24. Rao VH, Rai V, Stoupa S, et al. Tumor necrosis factor-alpha regulates triggering receptor expressed on myeloid cells-1-dependent matrix metalloproteinases in the carotid plaques of symptomatic patients with carotid stenosis. Atherosclerosis. 2016; 248:160–169. [PubMed: 27017522]

25. Pelham CJ, Pandya AN, Agrawal DK. Triggering receptor expressed on myeloid cells (TREM) receptor family modulators: a patent review. Expert Opin Ther Pat. 2014; 24(12):1383–1395. [PubMed: 25363248]

26*. Bostanci N, Ozturk VO, Emingil G, et al. Elevated oral and systemic levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in periodontitis. J Dent Res. 2013; 92(2):161–165. Comparative evaluation of salivary and serum levels of sTREM-1 in the presence of oral inflammation. [PubMed: 23242230]

27. Bostanci N, Belibasakis GN. Doxycycline inhibits TREM-1 induction by *Porphyromonas gingivalis*. FEMS Immunol Med Microbiol. 2012; 66:37–44. [PubMed: 22540741]

28. Wu M, Peng A, Sun M, et al. TREM-1 amplifies corneal inflammation after *Pseudomonas aeruginosa* infection by modulating toll-like receptor signaling and Th1/Th2-type immune responses. Infect Immun. 2011; 79:2709–2716. [PubMed: 21555403]

29. Pelham CJ, Agrawal DK. Emerging roles for triggering receptor expressed on myeloid cells receptor family signaling in inflammatory diseases. Expert Rev Clin Immunol. 2014; 10(2):243–256. [PubMed: 24325404]

30. Hajishengallis G. Too old to fight? Aging and its toll on innate immunity. Mol Oral Microbiol. 2010; 25:25–37. [PubMed: 20305805]

31**. Hall SC, Agrawal DK. Toll-like receptors, triggering receptor expressed on myeloid cells family members and receptor for advanced glycation end products in allergic airway inflammation. Expert Rev Respir Med. 2016; 10(2):171–184. Summation of the roles of TREM-1 during the phases of a host's immune response. [PubMed: 26678062]

32. Varanat M, Haase EM, Kay JG, et al. Activation of the TREM-1 pathway in human monocytes by periodontal pathogens and oral commensal bacteria. Mol Oral Microbiol. 2016 [Epub ahead of print].

33. Dong L, Zhou Y, Zhu ZQ, et al. Soluble epoxide hydrolase inhibitor suppresses the expression of triggering receptor expressed on myeloid cells-1 by inhibiting NF-kB activation in murine macrophage. Inflammation. 2016 [Epub ahead of print].

34*. Willi M, Belibasakis GN, Bostanci N. Expression and regulation of triggering receptor expressed on myeloid cells 1 in periodontal diseases. Clin Exp Immunol. 2014; 178:190–200. Experiment showing upregulation of TREM-1 mRNA in the presence of chronic and aggressive periodontitis. [PubMed: 24924298]

35*. Chen SS, Wang K, Zhao J, et al. Increased expression of triggering receptor expressed on myeloid cells 1 and 2 in inflamed human gingiva. J Periodont Res. 2016 [Epub ahead of print]. **Experiment showing significant increase in TREM-1 expression in gingival epithelium and subgingival connective tissue in the presence of oral inflammation.**

36. Pelham CJ, Agrawal DK. Emerging roles for triggering receptor expressed on myeloid cells (TREM) receptor family signaling in inflammatory diseases. Expert Rev Clin Immunol. 2014; 10(2):243–256. [PubMed: 24325404]

37. Ozturk VO, Belibasakis GN, Emingil G, et al. Impact of aging on TREM-1 responses in the periodontium: A cross-sectional study in an elderly population. BMC Infectious Diseases. 2016; 16:429. [PubMed: 27542376]

38. Hajishengallis G. Immune evasion strategies of *Porphyromonas gingivalis*. J Oral Biosci. 2011; 53(3):233–240. [PubMed: 22162663]

39. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012; 10(10):717–725. [PubMed: 22941505]

40. Johnson JD, Chen R, Lenton PA, et al. Persistence of extracrevicular bacterial reservoirs after treatment of aggressive periodontitis. J Periodontol. 2008; 79:2305–2312. [PubMed: 19053921]

41. Reddi D, Belibasakis GN. Transcriptional profiling of bone marrow stromal cells in response to *Porphyromonas gingivalis* secreted products. PLoS One. 2012; 7:e43899. [PubMed: 22937121]

42. Mysak J, Podzimek S, Sommerova P, et al. *Porphyromonas gingivalis*: Major periodontopathic pathogen overview. J Immunol Res. 2014; 2014:476068. [PubMed: 24741603]

43. Bostanci N, Thurnheer T, Aduse-Opoku J, et al. *Porphyromonas gingivalis* regulates TREM-1 in human polymorphonuclear neutrophils via its gingipains. PLoS ONE. 2013; 8(10):e75784. [PubMed: 24124513]

44. Liang S, Domon H, Hosur KB, et al. Age related alterations in innate immune receptor expression and ability of macrophages to respond to pathogen challenge in vitro. Mech Ageing Dev. 2009; 130:538–546. [PubMed: 19559723]

45. Bartold PM, Marshall RI, Hayes DR. Periodontitis and rheumatoid arthritis: A review. J Periodontol. 2005; 76(11):2066–2074s.

46. Crotti TN, Dharmapatni AA, Alias E, et al. The immunoreceptor tyrosine-based activation motif (ITAM)-related factors are increased in synovial tissue and vasculature of rheumatoid arthritic joints. Arthritis Res Ther. 2012; 14(6):R245. [PubMed: 23146195]

47**. Crotti CN, Dharmapatni A, Alias E, et al. Osteoimmunology: Major and costimulatory pathway expression associated with chronic inflammatory induced bone loss. J Immunol Res. 2015; 2015:281287. First discussion of the presence and possible functions of TREM-2 in periodontal diseases. [PubMed: 26064999]

48. Nguyen AH, Koenck C, Quirk SK, et al. Triggering receptors expressed on myeloid cells in cutaneous melanoma. Clin Translational Sci. 2015; 8(5):441–444.

49. Thankam FG, Dilisio MF, Dietz NE, et al. TREM-1, HMGB1 and RAGE in the shoulder tendon: Dual mechanisms for inflammation based on the coincidence of glenohumeral arthritis. PLoS One. 2016; 11(10):e0165492. [PubMed: 27792788]

50. Nguyen AH, Berim IG, Agrawal DK. Chronic inflammation and cancer: Emerging roles of triggering receptors expressed on myeloid cells. Expert Rev Clin Immunol. 2015; 11(7):849–857. [PubMed: 25954917]

51. Subramanian S, Pallati P, Sharma P, et al. TREM-1 associated macrophage polarization plays a significant role in inducing insulin resistance in obese population. J Translational Med. 2017; 15(1):85.

52. Subramanian S, Pallati P, Rai V, et al. Triggering receptor expressed on myeloid cells-1 is a novel biomarker for insulin resistance in the obese population. Obesity. 2017; 25(3):527–538. [PubMed: 28111922]

53. McMahon L, Schwartz K, Yilmaz O, et al. Vitamin D-mediated induction of innate immunity in gingival epithelial cells. Infect Immun. 2011; 79(6):2250–2256. [PubMed: 21422187]

54. Pandupuspitasari N, Khan F, Huang C, et al. Novel Attributions of TREMs in immunity. Curr Issues Mol Biol. 2016; 20:47–54. [PubMed: 26738206]

55. Gibot S, Buonsanti C, Massin F, et al. Modulation of the triggering receptor expressed on the myeloid cell type 1 pathway in murine septic shock. Infect Immun. 2006; 74(5):2823–2830. [PubMed: 16622220]

56. Sharif O, Bolshakov VN, Raines S, et al. Transcriptional profiling of the LPS induced NF-κB response in macrophages. BMC Immunol. 2007; 8:1. [PubMed: 17222336]

57. El Mezayen R, El Gazzar M, Seeds MC, et al. Endogenous signals released from necrotic cells augment inflammatory responses to bacterial endotoxin. Immunol Lett. 2007; 111:36–44. [PubMed: 17568691]

58. Gibot S, Kolopp-Sarda MN, Bene MC, et al. A soluble form of triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis. J Exp Med. 2004; 200:1419–1426. [PubMed: 15557347]

59. Bouchon A, Facchetti F, Weigand MA, et al. TREM-1 amplifies inflammation and is crucial mediator of septic shock. Nature. 2001; 410:1103–1107. [PubMed: 11323674]

Key Issues

- Periodontitis affects 40–50% of adults and has been linked to systemic inflammatory conditions.

- Bacteria in biofilms are especially protected against a host's immune response which can lead to a chronic hyperinflammatory state and significant periodontal tissue destruction.

- TREM-1 is found in gingival epithelial and subepithelial connective tissues.

- TREM-1 expression is significantly upregulated in the presence of oral inflammation.

- *P. gingivalis* can upregulate TREM-1 expression, but this effect can be reversed by subantimicrobial doses of Doxycycline.

- *P. gingivalis* is able to dual-regulate immune responses to evade destruction by host cells while gathering nutrients from host fluids.

- There is a positive correlation between sTREM-1 levels in GCF in the presence of inflammation.

- There is a positive correlation between salivary and serum sTREM-1 levels indicating a possible connection between oral and systemic inflammation.

- While the role of TREM-2 is not fully understood, there is increased expression during periodontal inflammation which could lead to increased osteoclastogenesis and bone destruction.

- More research needs to be conducted regarding TREM-1, sTREM-1, and TREM-2 and their involvement in periodontitis.
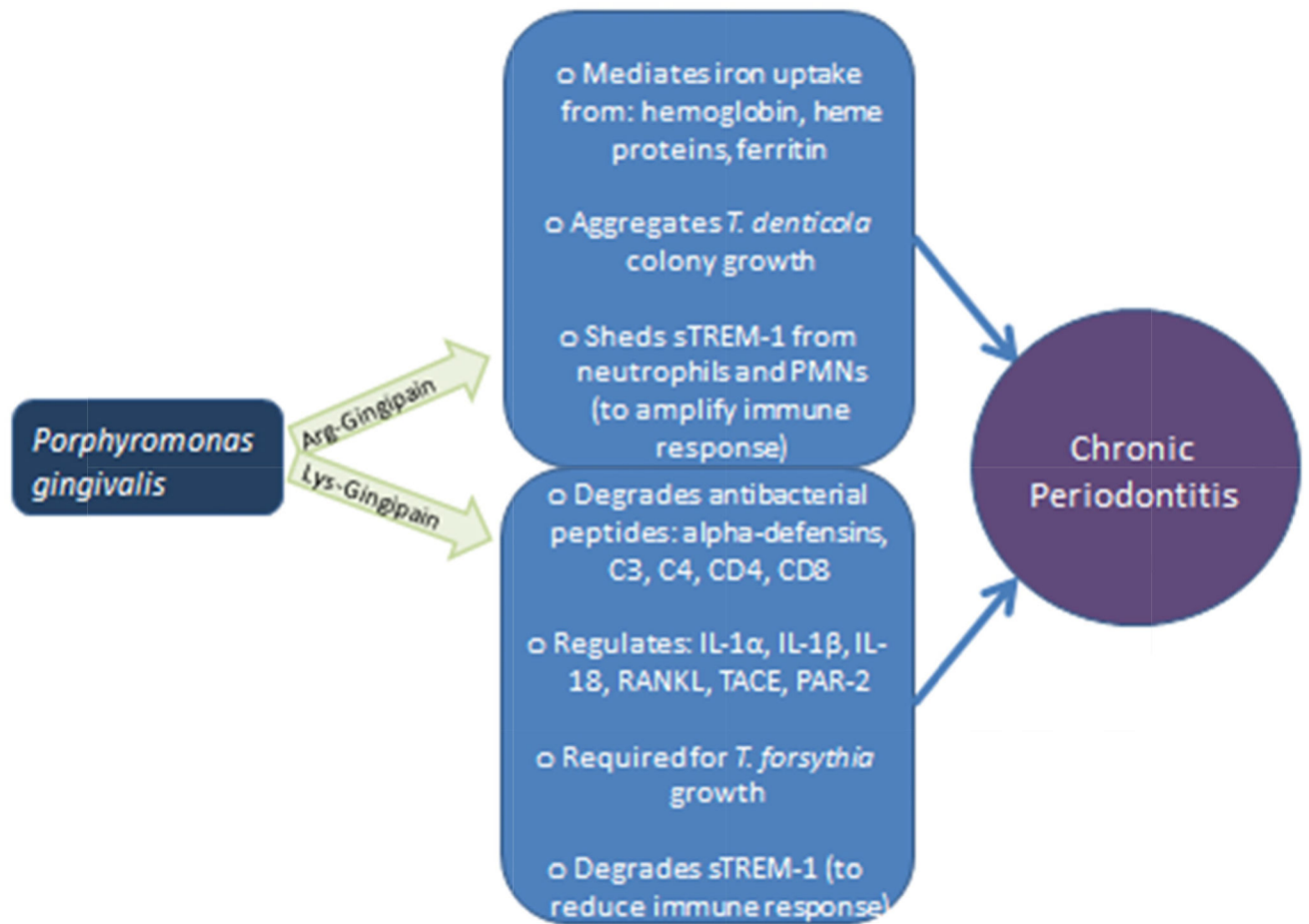
Figure 1.
Schematic diagram showing *P. gingivalis* dual immunoregulatory pathways.
C3/C4: Complement component 3/4, **CD**: Cluster of differentiation, **IL**: Interleukin, **PAR-2**: Protease activated receptor-2, **PMNs**: Polymorphonuclear neutrophils, **RANKL**: Receptor activator of nuclear factor kappa-B ligand, **TACE**: TNF-α converting enzyme.

Table 1

Effect of TREM-1 Amplification on Inflammatory Mediator Expression

TREM-1 Amplification		
Modifier	**Outcome**	**Source**
Unknown natural ligand	Increases IL-1α, TNF-α, GM-CSF, IL-8, MCP-1, MCP-3, MIP-1	Bisson et al. 2012 [21]
Bacteria (P. aeruginosa, S. aureus, S. pneumoniae)	Increase TREM-1 expression on cell surfaces	Bisson et al. 2012 [21]
Unspecified	Increases neutrophil degranulation and phagocytic respiratory burst	Gibot et al. 2006 [55]
TLRs (especially TLR-2 and TLR-4); Agonist monoclonal antibodies; NLRs	Synergistically increase TNF-α, IL-1β, and inhibit IL-10, cause excessive inflammation	Paknejad et al. 2016 [17] Dong et al. 2016 [33]
TLR-2	Upregulates TREM-1 expression via MYD88-dependent pathway	Varanat et al. 2016 [32]
Unspecified	Increases IL-1β	Bostanci et al. 2011 [8]
Unspecified	Enhances neutrophil phagocytosis and degranulation	Hajishengallis et al. 2010 [30]
Active Vitamin D [1,25(OH)$_2$D3]	Increases TREM-1 expression	McMahon et al. 2011 [53]
Anti-TREM-Antibody	Stimulates NF-κB regulated immune response (TNF-α, IL-2, IL-12p40, IL-1β)	McMahon et al. 2011 [53] Sharif et al. 2007 [56]
LPS and Peptidoglycan	TREM-1 upregulation	Bostanci and Belibasakis 2012 [27] Dong et al. 2016 [33]
TGF-β1	TREM-1 upregulation	Dong et al. 2016 [33]
Unspecified	Increases expression of cell surface proteins: CD11, CD29, and CD80	Pandupuspitasari et al. 2016 [54]
HMGB-1 and HSP70	May be natural ligands for TREM-1	El Mezayen et al. 2007 [57]

IL: Interleukin, **TNF**: Tumor necrosis factor, **GM-CSF**: Granulocyte-macrophage colony-stimulating factor, **MCP**: Monocyte chemoattractant protein, **MIP**: Macrophage Inflammatory Proteins, **TLR**: Toll-like receptor, **NLR**: NOD-like receptor, **NF-κB**: Nuclear factor kappa-light-chain-enhancer of activated B cells, **LPS**: Lipopolysaccharide, **TGF**: Transforming growth factor, **CD**: Cluster of differentiation, **MHC**: Major histocompatibility complex, **HLA-DR**: Human leukocyte antigen - antigen D Related, **HMGB-1**: High-mobility group box protein-1, **HSP**: Heat shock protein.

Table 2

Effect of TREM-1 Suppression on Inflammatory Mediator Expression

TREM-1 Suppression		
Modifier	**Outcome**	**Source**
Unspecified	Decreases: IL-1β, IFN-γ, and IL-6 Increases: IL-4, IL-5, and IL-10	Wu et al. 2011 [28]
Unspecified	Blocks cytokine production	Pelham et al. 2014 [25]
Porphyromonas gingivalis	Shifts from cell surface expression of TREM-1 to soluble form (sTREM-1). Effect can be abrogated by Doxycycline*	Liang et al. 2009 [44] Bostanci and Belibasakis 2012 [27]
LP-17	Inhibits IL-6 and TNF-α expression in macrophages	Cui et al. 2013 [7]
Inhibition of NF-κB	TREM-1 downregulation	Dong et al. 2016 [33]
Unspecified	Decreases expression of key proteins in the TLR-4 signaling pathway. Decreases downstream molecules of the NF-κB pathway.	Pelham et al. 2014 [25]
IL-10	Abrogates TREM-1 upregulation due to TLR stimulation	Hall and Agrawal 2016 [31]
sTREM-1	Potentially: downregulates TREM-1 signaling pathway by competitively binding the natural ligand of TREM-1	Bouchon et al. 2001 [59]

IFN: Interferon, **IL**: Interleukin, **NF-κB**: Nuclear factor kappa-light-chain-enhancer of activated B cells, **TLR**: Toll-like receptor, **TNF**: Tumor necrosis factor.