

The role of the IL-33-ST2 axis in the pathogenesis of periodontitis

O papel do eixo IL-33-ST2 na patogênese da periodontite

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ABSTRACT | INTRODUCTION: Periodontitis is a chronic inflammatory disease that arises from an interaction of local dysbiosis in the subgingival biofilm and the host immune response. The disease triggers an inflammatory process that can cause periodontal tissue breakdown. In addition, the production of inflammatory mediators can negatively affect other areas of the body and influence the development and/or severity of associated disorders, including asthma, diabetes and cardiovascular diseases. **OBJECTIVE:** Conduct a literature review to comprehensively investigate the role of ST2 and IL-33 and the contribution of the IL-33 / ST2 axis in the pathogenesis of periodontitis. **METHODS:** It is a narrative literature review, which used the databases Pubmed and Academic Google with a total of 114 results. **RESULTS:** A range of immunological markers has been shown to be relevant in the immunopathogenesis of periodontitis, like cytokines of the interleukin 1 (IL-1) family, such as IL-1 β and IL-33. IL-33, through its ST2 receptor, appears to be involved in the development of periodontitis, acting as a molecule that signals tissue damage from infection and also emits an endogenous signal that activates the immune response and / or worsens bone resorption by activating osteoclastogenesis, in a manner dependent or not on RANKL. **CONCLUSION:** The diversity of the methodologies of these studies hampers attempts to standardize and make correlations between published data, in this sense, the IL-33 / ST2 axis remains a relevant topic in investigations focused on the diagnosis and treatment of periodontal disease.

KEYWORDS: Periodontal disease. Immune response. Cytokines. Dysbiosis.

RESUMO | INTRODUÇÃO: A periodontite é uma doença inflamatória crônica que surge de uma interação entre a disbiose local no biofilme subgingival e a resposta imune do hospedeiro. A doença desencadeia um processo inflamatório que pode causar ruptura do tecido periodontal. Além disso, a produção de mediadores inflamatórios pode afetar negativamente outras áreas do corpo e influenciar o desenvolvimento e / ou gravidade de distúrbios associados, incluindo asma, diabetes e doenças cardiovasculares. **OBJETIVO:** Realizar uma revisão da literatura para investigar o papel de ST2 e IL-33 e a contribuição do eixo IL-33 / ST2 na patogênese da periodontite. **MÉTODOS:** Trata-se de uma revisão narrativa da literatura, que utilizou as bases de dados Pubmed e Academic Google em um total de 114 resultados. **RESULTADOS:** Uma gama de marcadores imunológicos têm se mostrado relevante na imunopatogênese da periodontite, como citocinas da família da interleucina 1 (IL-1), IL-1 β e IL-33. A IL-33, por meio de seu receptor ST2, parece estar envolvida no desenvolvimento da periodontite, atuando como uma molécula que sinaliza o dano tecidual da infecção e também emite um sinal endógeno que ativa a resposta imune e / ou piora a reabsorção óssea pela ativação da osteoclastogênese, tanto por aumentos em RANK-L como por redução de OPG, ou independentemente dessa via. **CONCLUSÃO:** A diversidade de metodologias desses estudos dificulta tentativas de padronização e correlações entre os dados publicados, nesse sentido, o eixo IL-33 / ST2 continua sendo um tema relevante nas investigações voltadas para o diagnóstico e tratamento da doença periodontal.

PALAVRAS-CHAVE: Doença periodontal. Resposta imune. Citocinas. Disbiose.

Introduction

Periodontal diseases are one of the most prevalent inflammatory diseases found in humans. Periodontal diseases can be seen in up to 90% of the global population¹, being the most common oral disease. It has been estimated that just 17.8% of the Brazilian adult population, aged between 35 and 44 years, has no periodontal disease. Gingivitis and periodontitis are among the most common periodontal diseases, while gingivitis is an inflammatory process that only affects the soft tissues around the teeth, periodontitis also affects supporting tissues. Periodontitis, an inflammatory, chronic, multifactorial disease occurring in periodontal tissue characterized by the progressive destruction of periodontal tissues (gingiva, alveolar bone and periodontal ligament), initiates from the presence of biofilm in the subgingival region². This arises from an imbalance between the microbiota and the host immune response, which triggers a dysbiotic process, which has the ability to modulate the host's response in a way that impairs immune surveillance and alters the balance, homeostasis³.

Microorganisms present in the subgingival biofilm, such as *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Prevotella intermedia*, may elicit an immune response in the host that can lead to serious systemic effects⁴. Previous studies have pointed to the impact of periodontal infection on systemic conditions, including cardiovascular disease, cerebrovascular accidents⁵, preterm birth and low neonatal birthweight⁶, in addition to diabetes⁷, asthma and pneumonia⁸.

One of the more widely accepted biological mechanisms linking this oral disease with systemic

conditions is the production of inflammatory markers. Several markers have been determined to play relevant roles in the development and/or progression of periodontitis, such as interleukin-6 (IL-6), tumoral necrosis factor (TNF) and IL-1 β ⁹. Others, such as IL-33, an interleukin of the IL-1 β family, and its receptor, ST2, have been poorly investigated. The participation of the IL-33/ST2 axis was recently shown to play an important role in the immunopathogenesis of periodontitis¹⁰.

IL-33 can act as an immune system alarmin (alarm signal) when released by damaged cells, which in turn activates other cells expressing the ST2 receptor. This can induce the release and production of inflammatory cytokines and may activate osteoclastogenesis, either dependently, or independently, of the RANK/ RANKL pathway. However, this process requires further investigation¹⁰.

Gene ST2, originally abbreviated as "serum STimulation-2" has come to be known as "suppression of tumorigenicity 2," IL-1RL1, IL-33R or T1. The suppressive effect of this gene was identified at the beginning of the growth cycle of quiescent BALB/c-3T3 cells, while also simultaneously promoting the growth of cells during cellular division. At the time, the ST2 ligand had not been identified¹¹.

It was later suggested that the ST2 gene encodes type I transmembrane protein ST2L (serum STimulation-2 large), which bears a sequence quite similar to that of the interleukin-1 receptor type I (IL-1RL1). Following this discovery, investigations began to focus on the immunological aspects of ST2. IL-33 was soon after identified as a member of the IL-1 family, and has been described as the only ligand to ST2¹².

IL-33 is considered to be a damage-associated molecular pattern (DAMP), as it is released by fibroblasts, macrophages and/or endothelial and epithelial cells in response to cellular injury or infection. IL-33 can also induce the activation of Th2 cells, which then produce IL-5 and IL-13, in addition to promoting the migration of neutrophils in an ST2-dependent manner. In eosinophils, this cytokine may induce degranulation, as well as the production of oxygen reactive species (ROS) and IL-5 secretion. In mastocytes, it promotes the release of IL-4, IL-5 and IL-6, while developing an M2 phenotype in macrophages. In contrast, IL-33 may induce the release of cytokines, such as IFN- γ and TNF, involved in inflammatory processes and diseases such as asthma, allergies, cardiovascular disease, inflammatory intestinal disorders, diabetes and periodontitis¹³.

Accordingly, the present study aimed to review the role of IL-33, its ST2 receptor and the IL-33/ST2 axis in an attempt to elucidate the influence of these mediators within the context of the pathogenesis of periodontitis.

Methods

It is a narrative literature review, which used the databases Pubmed and Academic Google. The search was carried out in March 2018 and the free search strategy was defined, using the descriptors: "IL33 and periodontal disease", "IL33 and periodontitis", "ST2 and periodontal disease", "ST2 and periodontitis". The inclusion criteria applied were: scientific articles published in the last twenty years, addressing the proposed topic, in Portuguese, English and Spanish. The following criteria were used to exclude articles: articles that were not related to the proposed theme, repeated articles and in languages other than English,

Portuguese and Spanish. The search revealed a total of 114 results: 43 found in google academic and 71 found in PubMed, with 43 duplicates. Finally, 43 articles were selected in accordance with the inclusion and exclusion criteria, based on the title and abstract. All selected articles are written in the English language

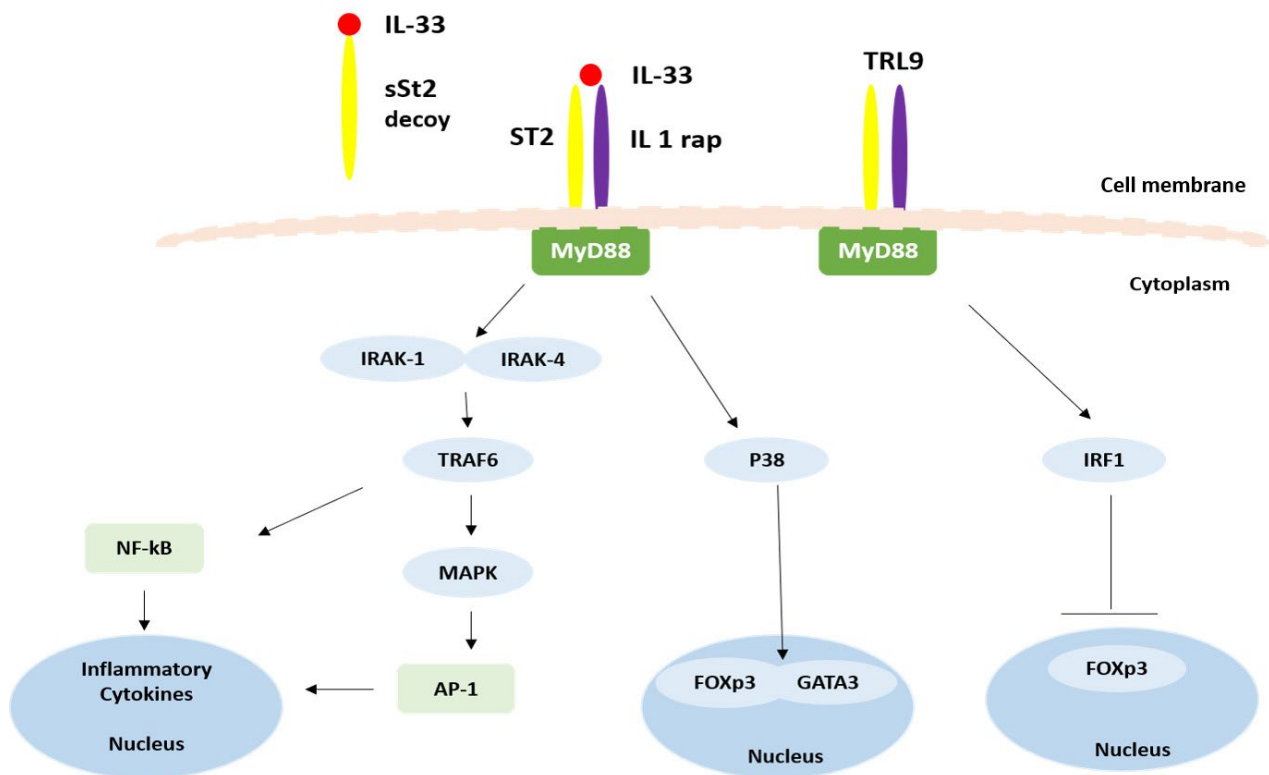
Results

The ST2 Receptor

The ST2 receptor was considered an orphan for many years until IL-33 was identified as the specific ligand of membrane-anchored ST2- ST2L12. Due to its structural characteristics, especially the presence of the intracytoplasmic domain known as Toll/interleukin-1 receptor (TIR), this receptor was also classified as a member of the IL-1 receptor superfamily. In addition, its molecular structure contains an extracellular domain that binds to IL-33 with the aid of Interleukin 1 Receptor Accessory Protein (IL-1RAP)¹⁴.

In general, after binding to IL-33, ST2L forms a heterodimer with IL-1RAP14 leading to the dimerization of the TIR domain. This intracellular signaling cascade then proceeds to recruit MyD88, the ligand protein of the TIR domain, and later activates the kinase associated with IL-1R (IRAK) and TNF receptor-associated factor 6 (TRAF6). Activated IRAK and TRAF6 can thus phosphorylate nuclear factor kappa B (NF- κ B) or Activator protein 1 (AP-1). The ST2/IL-33 signaling pathway may culminate either by way of the activation of AP-1 (through mitogen-activated protein kinases – MAPK), or NF- κ B. However, previous investigations focused on this topic were unable to clarify this phenomenon, yet did establish that the activation of both transcription factors leads to the production of inflammatory cytokines¹⁵.

Figure 1. The ST2/IL-33 signaling pathway



Source: Adapted from GRIESENAUER and PACZESNY (2017). After the binding of IL-33 with ST2 / IL-1, a heterodimer is formed with IL 1RAP that will recruit MyD88, the TIR domain binding protein, and later activate the IL-1R-associated kinase (IRAK) and factor 6 associated with the TNF receptor (TRAF6). Activated IRAK and TRAF6 will phosphorylate nuclear factor kappa B (NF-kB) or Activator protein 1 (AP-1). The ST2 / IL-33 signaling pathway can be through the activation of AP-1 (through mitogen-activated protein kinases - MAPK) or NF-kB. The activation of both transcription factors leads to the production of inflammatory cytokines

In regulatory T-cells (Treg), the ST2/IL-33 axis can promote the expression of a member of the forkhead family of protein transcription factors (Foxp3) and the ligand GATA3 while simultaneously promoting the proliferation of Treg cells mediated by transforming growth factor (TGF- β 1) by way of a mechanism dependent on the P38 protein. Since this effect is observed in Tregs in a microenvironment independent of external inflammatory responses, it is believed that the ST2/IL-33-GATA3-Foxp3 pathway is canonical. On the other hand, in a non-canonical MyD88-dependent pathway signaling by the IFN type-1 regulatory factor (IRF) can inhibit the activation of Tregs by way of binding to the Foxp3 promoter, which interrupts the transcription of Foxp3 in T cells, thus favoring the inflammatory response¹⁶.

It was initially suggested that ST2L was correlated with the Th2-mediated immune response, since this ligand is preferentially expressed in murine and human Th2 cells regardless of stimulation by cytokines, e.g. IL-4, IL-5 e IL-10, yet this expression is supposedly absent in Th1 cells. However, it is now known that this receptor is also expressed by a variety of other cells, including Th1 cells, B lymphocytes, neutrophils, monocytes, mastocytes, dendritic cells, basophils, eosinophils, natural killer (NK) cells, NK T cells (NKT), type 2 innate lymphoid cells (ILC2) and Tregs¹⁷.

Additional research has demonstrated that the ST2 gene also produces, via alternative splicing, a secreted protein form with an extracellular domain identical to that of ST2L, soluble ST2 (ST2s) (HAYAKAWA H, et al., 2007) and two variant forms: ST2V (variant) and ST2LV (large variant). ST2V is expressed in leukemic human cells and is highly expressed in gastrointestinal organs ST2LV is secreted by tissues in the eyes, heart, lungs and liver, and is present during posterior development of embryogenesis¹⁸.

The soluble form of the protein, ST2s, is not known to trigger an intracellular cascade, but is capable of binding to soluble IL-33, which prevents this cytokine from interacting with the ST2L receptor, thereby blocking the effects generated by this interaction¹⁹. It is in this context that soluble ST2 has been described as an antagonist receptor against the effects of IL-33²⁰ as it modulates the participation of this cytokine in the inflammatory process.

In humans, ST2s may be spontaneously produced not only by cells in the lungs, kidneys, heart and small intestine, but also by mastocytes activated by IL-33. ST2s production is increased in epithelial cells of the human lung and cardiac myocytes under stimulation by cytokines such as IL-1 β and TNF. In addition, increased levels of ST2s may inhibit the production of cytokines, such as IL-4 and IL-5, in contrast to what is observed in cytokines IFN- γ and IL-2²¹.

Elevated serum levels of ST2s have been reported, as well as in other body fluids, in individuals who suffer from a variety of disorders, such as bronchial asthma, systemic lupus erythematosus, acute eosinophilic pneumonia, celiac disease, idiopathic pulmonary fibrosis, malignant pleural effusion, subarachnoid hemorrhage, HIV progression and acute myocardial infarction²².

It is impossible to explain the wide spectrum of diseases in which elevated levels of ST2s are present in the serum or other body fluids as simply the result of the immunologic functioning of proteins coded by the ST2 gene. Although still in need of clarification, it seems that ST2-produced proteins influence cellular proliferation, growth and migration via a pathway independent of IL-33 binding, which has been demonstrated in both malignant cells and also in a strain of murine fibroblasts²³.

The Interleukin 33

IL-33 was firstly identified as a protein present in the high endothelial venules (HEV) of the tonsils, Peyer's plaques and lymph nodes, being described as a "nuclear factor of the high endothelial venules" (NF-HEV). It is now known that IL-33 can act as an extracellular ligand or as an intracellular signaling molecule, accordingly, this protein's characteristics are unconventional in the interleukin family.

For instance, while IL-33 is not actively secreted by cells²⁴ it reaches the extracellular environment by cellular injury or death.

Extracellular IL-33 plays a role in the coordination of immunological defense and repair mechanisms. Intracellular IL-33 participates in the regulation of gene expression as a nuclear protein, in addition to functioning as a molecule, known as "alarmin", that recognizes cellular damage. To this end, IL-33 accumulates in the nuclei of cells and is released following stress or damage arising from cellular or tissue injury, infection or necrosis. In these situations, IL-33 acts as an alarmin, it alerts the immunological system to the presence of trauma or infection. The term alarmin pertains to endogenous molecules that emit this type of signal, such as those belonging to the family of DAMPs – damage-associated molecular patterns²⁵.

Since it is constitutively expressed in the cellular nucleus, IL-33 is immediately available for release into the extracellular environment after damage. This protein is also present in a range of immune cells, e.g. monocytes/macrophages and dendritic cells, and non-immune cells, such as fibroblasts, adipocytes, epithelial cells, endothelial cells, smooth muscle cells and salivary gland cells, as well as in a variety of tissues, including stomach, lung, spinal medulla, cerebral and skin²⁶.

When secreted, this cytokine is capable of activating cells possessing the ST2 receptor, such as basophils, mastocytes, eosinophils, type 2 innate lymphoid cells (ILC2), Th2 lymphocytes and Tregs²⁷.

It is also known that IL-33, in spite of its full-length activation undergoes cleavages in its original form. Cleavage by inflammatory proteases generates mature forms that significantly increase the activity of this cytokine. The mature forms generated by neutrophil and mastocyte proteases may be the principal bioactive forms of IL-33 in diseased tissues²⁸. It has been demonstrated that IL-331-270 undergoes proteolytic cleavage by cathepsin G and neutrophil-derived elastase, which produces the following peptides: IL-3395-270, IL-3399-270 and IL-33109-270, all of which present greater biological activity than their precursor IL-331-270. It is interesting to note that after IL-33 undergoes the proteolytic cleavage, it is unable to translocate to the nucleus²⁹.

As IL-33 is a cytokine with multiple functions, its action varies in accordance with the type of stimulus encountered. It can function as a transcription factor due to some of its structural characteristics, such as a nuclear localization sequence in the N-terminal domain and a homeodomain containing a helix-turn-helix motif, where the IL-33-containing domains overlap with high local concentrations of DNA, indicating association of endogenous IL-33 with heterochromatin, thus, it is capable of binding to heterochromatin in the nucleus of endothelial cells *in vivo* and can exert a regulatory transcriptional function that remains poorly understood³⁰.

When tissue damage is accompanied by infiltrating or commensal microorganisms, as occurs in the intestinal epithelium, the action of IL-33 becomes mediated by the induction of IFN- γ and TNF. This occurs when antigen-presenting cells (APC) activated by pathogen-associated molecular patterns (PAMP) secrete IL-12 and IL-23. IL-12 increases ST2 expression in T and NK cells, which, when bound to IL-33, forms the IL-33/ST2 axis, inducing increases in IFN- γ and TNF. It is important to note the inflammatory process is accompanied by a regulatory process. Accordingly, elevated levels of IFN- γ and TNF can promote the secretion of an IL-33 antagonist, soluble ST2, with respect to IL-23, this cytokine neutralizes IL-33 signaling in Treg ST2+ cells and can limit the proliferating capability of IL-33 in Treg ST2+ cells³¹ thereby contributing to the exacerbation of the inflammatory response.

As in all pathways of immune response induction, as the etiological agent becomes controlled, negative feedback mechanisms are activated to return the involved tissues to equilibrium. Accordingly, once PAMPs and/or pathogens are eliminated, IL-33 production at the site of infection initiates a reparative tissue process via ILC2 and Treg. IL-33 signaling in T cells stimulates the Treg response both initially, by enhancing Treg cell differentiation mediated by TGF- β 1, and later by providing the necessary signaling to maintain Treg cells at the sites of inflammation³².

In allergic or parasitic processes, IL-33 stimulates cells, such as basophils, mastocytes, ILC2s and Th2

lymphocytes to produce IL-5, IL-13, chemokines and proteins that act as chemo attractants for eosinophils²⁶. While these cells mediate parasite elimination, the chronic activation of this pathway can cause allergic disease and tissue fibrosis. Under these conditions, IL-33 stimulates myeloid cells to secrete IL-2, which synergizes with IL-33 to stimulation the expansion of ST2+ Treg cells³³.

ST2+ Treg cells, present in the primary and secondary lymphoid tissues, adipose tissue, the lungs and intestine, stimulate immunological regulatory function and the tissue repair process in response to IL-33 by way of cellular proliferation and the expression of amphiregulin epidermal growth factor (AREG). IL-33 is also capable of activating ILC2, which aids in tissue repair through the production of AREG and the differentiation of M2 macrophages²⁸.

As previously mentioned, IL-33 acts not only on tissue regulation and repair mechanisms but may also participate in inflammation. In sum, IL-33 may be involved in the triggering of inflammatory processes through the induction of IFN- γ , TNF, IL-6 and IL-1 β expression or by interfering in the activation of Treg, ILC2 or M2 macrophage cells²⁸.

It is interesting to note that the action of IL-33 is regulated by several mechanisms: the retention of this cytokine in the nuclei of IL-33-producing cells during homeostasis the cleavage of domains similar to IL-1 by caspases during apoptosis, through the sequestration of IL-33 by soluble ST2 protein, through the action of single Ig IL1R-related (SIGIRR) molecule in the formation of dimers with ST2L, by the oxidation of cysteine residues and the formation of disulfide (S-S) bridges in IL-33 after extracellular release³⁴.

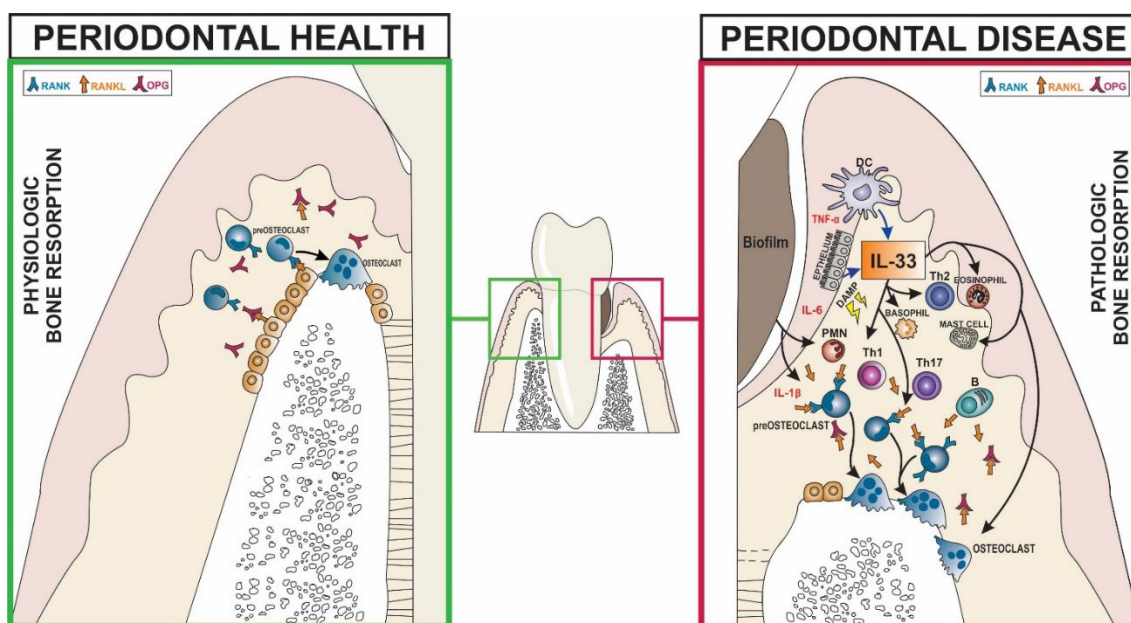
Finally, the participation of the IL-33/ST2 axis has already been described in the immunopathogenesis of several disorders, such as cardiovascular disease, asthma, atopic dermatitis, inflammatory bowel disease, systemic erythematosus lupus, allergic rhinitis, chronic obstructive pulmonary disease and chronic colitis as well as in destructive inflammatory disorders, such as rheumatoid arthritis and periodontitis³⁵.

The IL-33/ST2 Axis in Periodontitis

Periodontitis is a disease with an etiology consisting of multiple bacteria, that trigger a range of immunoinflammatory events, leading to tooth loss. While it is more prevalent in adults, this disease can also beset children and adolescents. The degree of tissue destruction is generally proportional to the presence of biofilm, intensity of host response related risk factors, It has been demonstrated that elevated levels of production and expression of IL-33 and its ST2L receptor are present in tissues and gingival epithelial cells in both mice and humans³⁶ providing further evidence of the role of the IL-33/ST2 axis in the pathogenesis of periodontitis.

IL-33 can be involved in the pathogenesis of periodontitis by way of several mechanisms (Figure 2). This cytokine can act as an alarmin at the moment of periodontal tissue damage, exacerbating local or systemic inflammatory processes through the activation of immune cells to secrete cytokines by way of the activation of the IL-33/ST2 axis and/or through the induction of osteoclastogenesis³⁷.

Figure 2. Role of IL-33 in the immunopathogenesis of periodontitis



Source: Author himself. Left: Representation of physiological bone resorption of periodontal tissues in the absence of periodontitis. Among other factors, bone resorption and formation are regulated by relative concentrations of RANKL (expressed by a variety of cells, including osteoblasts), the RANK receptor in pre-osteoclasts and the soluble receptor OPG. The binding of RANKL to the pre-osteoclast induces its differentiation into multinucleated cells that become mature osteoclasts. When OPG concentrations are relatively higher than RANKL expression, OPG binds to RANKL, thereby inhibiting the binding of this molecule to RANK, leading to reduced osteoclast formation (osteoclastogenesis) and to the apoptosis of pre-osteoclasts. Right: Representation of bone resorption in periodontal tissues when periodontal pathogens colonize the oral microenvironment, inducing local dysbiosis, inflammatory reactions and cellular damage in surrounding tissues. When cells are damaged, IL-33 is released by epithelial cells and fibroblasts, acting as alarmin (DAMP) that signals immune system activation. Immunological cells, such as polymorphonuclear neutrophils (PMN), B cells (B), dendritic cells (DC) and T cells (Th1, Th2 and Th17) are recruited to the site of inflammation. The production of cytokines (TNF, IL-1b, IL-6 and IL-33) becomes increased in the microenvironment, enhancing the expression of RANKL by osteoclasts. IL-33 is constitutively expressed by dendritic cells and the monocytes. Activated T cells also secrete soluble RANKL and TNF, which induces the release of IL-33 in lysed gingival fibroblasts. Once released, IL-33 is capable of activating cells, such as basophils, mastocytes, eosinophils, innate type 2 lymphoid cells (ILC2), Th2 lymphocytes and Tregs, in addition to increasing the production of RANKL and decreasing the production of OPG, thus favoring RANK-RANKL interaction. Periodontal pathogens can also induce the expression of IL-33 and RANKL, which contributes to increased osteoclastogenesis and, consequently, alveolar bone loss. In addition, IL-33 can directly bind to its receptor, ST2, in the osteoclasts and activate these cells. This activation, differently than the abovementioned RANK/RANKL mechanism, occurs via the IL-22/ST2 axis.

It is believed that proinflammatory cytokine levels become elevated due to infectious bacterial processes³⁷. Keystone pathogens present in the dysbiotic biofilm, such as *Porphyromonas gingivalis*, can induce the expression of IL-33 in human gingival and oral epithelial cells by way of gingipain-dependent mechanisms. In dendritic murine cells, TLR2-mediated IL-33 expression primarily increases in response to stimulation with fimbriae and lipopeptides derived from *P. gingivalis*. Immature dendritic cells, such as Langerhans cells, are known to infiltrate the gingival epithelium of individuals with gingivitis or periodontitis. These findings suggest that the gingival epithelium represents an important source of IL-33 production following infection by periodontal pathogens and that IL-33 can modulate the function of dendritic cells in the context of periodontal disease³⁸.

Of the diverse cells activated by the IL-33/ST2 axis, Th2 lymphocytes and, in particular, mastocytes exert relevant roles in the pathogenesis of periodontitis. Th2 lymphocytes are important in later stages of periodontitis, as these cells produce IL-4, IL-5 and IL-13 cytokines. Mastocytes are present in all tissue types, immediately recognize IL-33 and are readily capable of inducing an inflammatory response³⁹. IL-33 can promote mastocyte survival, maturation and maintenance, as well as initiate the expression of chemokines, IL-6, IL-1 β TNF and lipid mediators.

TNF has been implicated as a powerful inducer of IL-33 production in lysed gingival fibroblasts, acting as an alarmin for this cytokine. As previously mentioned, following the elimination of pathogenic agents, IL-33 exerts a regulatory and repairing effect on periodontal tissue by inducing the production of cytokines, such as IL-4, IL-5, IL-6, IL-10 e IL-13. In addition, inflammatory cytokines present in the gingival fluid of individuals with periodontitis may stimulate the production of RANKL, a protein known to be crucial to osteoclastogenesis⁴⁰.

Alveolar bone loss is a characteristic sign of periodontitis. Osteoclastogenesis mediated by the activating receptor of NF- κ B (RANK) and its ligand (RANKL) is essential to the bone resorption process. RANK is expressed on the surfaces of osteoclasts, while RANKL is expressed on the surfaces of osteoblasts. During binding, osteoclastic precursor differentiation and activation signaling becomes

activated, inducing the bone resorption observed in periodontitis. Osteoprotegerin (OPG) is a soluble receptor that, when bound to RANKL, impedes this ligand's interaction with RANK and is capable of antagonizing the effects of binding, thereby preserving bone integrity. However, the role of IL-33 in the induction of RANKL production as well as the reduction of OPG production must be noted, since this activity favors the RANK/RANKL interaction that activates osteoclastogenesis and consequent alveolar bone loss (Figure 2)⁴¹.

In mice infected with *P. gingivalis*, treatment with IL-33 significantly increased alveolar bone loss, with attenuated alveolar loss observed in mice knocked out for ST2. In addition, increased IL-33 expression was demonstrated in human and mouse models of periodontitis¹⁰. Furthermore, it was shown that both *P. gingivalis* and IL-33 were able to independently induce RANKL expression, further emphasizing the relevant role of IL-33 in the pathogenesis of periodontitis, studies have shown that overexpression of IL-33 in gingival epithelial cells is associated with periodontitis and can trigger the expression of RANK-L, in addition to the direct effect of *P. gingivalis*, therefore, IL-33 can act as an extracellular alarm, showing properties pro-inflammatory in periodontitis, perpetuating bone resorption induced by *P. gingivalis* infection³⁶.

IL-33 was also demonstrated to stimulate osteoclast differentiation in vitro by way of increasing the production of osteoclast transcription factors. This activation occurs via the bonding of IL-33 to the ST2 receptor, regardless of the activation of the RANK/RANKL axis, which differs from the mechanism described in the previous paragraph⁴².

It was further observed that the total amount of IL-33 detected in gingival fluid seemed to be greater in individuals with periodontitis, in addition to being positively correlated with gingival and plaque index clinical parameters¹⁰. However, this was not the case with IL-33 levels in saliva and plasma, since no variations were observed between individuals with or without periodontitis or gingivitis in comparison to healthy individuals, although, without statistical relevance, IL-33 levels were higher in clinically healthy patients. This could be explained by IL-33 levels below the detection range of the quantification method employed, or, simply, due to a lack of association with periodontitis⁴³.

Although few studies have demonstrated a relationship between the presence of the IL-33/ST2 axis and the pathogenesis of periodontitis, this warrants further investigation.

Conclusion

The pleiotropic cytokine IL-33 is capable of eliciting action from a diverse range of cell types, both those of innate immunity and adaptive response, through binding with ST2. Although the IL-33/ST2 axis may be involved in the development of a variety of diseases through the release of inflammatory mediators, it can also perform relevant functions in the regulatory and tissue repair phases. In the pathogenesis of periodontitis, the role played by this axis in osteoclastogenesis and the consequent exacerbation of bone loss, in a manner dependent or not on RANKL, is notable. In addition, IL-33 can function as a DAMP, as well as a cytokine that activates cells, such as mastocytes and Th2 lymphocytes. Previous studies on IL-33 have classified periodontitis differently, and employed the collection of dissimilar samples. The diversity of these studies' methodologies hampers attempts to standardize and make correlations among the published data, which consequently hinders our ability to draw conclusions regarding the role of the IL-33/ST2 axis in periodontitis. Accordingly, the IL-33/ST2 axis remains a relevant topic in investigations focused on the diagnosis and treatment of periodontal disease.

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Author contributions

Lopes MPP drafted and developed the review. Carvalho-Filho PC, Santos-Lima EKN, Santos RPB, Gomes-Filho IS participated in writing and in the critical review. Trindade SC participated in writing and in the critical review and supervised the research.

Competing interests

No financial, legal or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

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