Association between pro-inflammatory cytokine interleukin-33 and periodontal disease in the elderly: A retrospective study

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Abstract:
Background: Senescence is a multifactorial process that in humans may be accompanied by inflammation and immune dysfunction in the oral cavity. Notably, periodontal disease, considered one of the most common inflammatory disorders in the oral cavity, has also been linked to the onset of other chronic inflammatory diseases common in the elderly. Thus, investigating immunity and inflammation during senescence may not only illuminate the pathophysiology of periodontal disease, but also identify new therapeutic targets. Materials and Methods: To this end, we retrospectively and systematically reviewed studies of immune molecules associated with periodontal disease. These studies were identified in PubMed from three independent searches based on distinct sets of search terms. Results: The data highlight the need to further investigate inflammatory molecules involved in chronic periodontal disease in the elderly, but strongly suggest that interleukin (IL)-33 is involved. Indeed, various genetic and environmental factors appear to contribute to pathogenesis via IL-33. Conclusion: The IL-33 axis may be promising therapeutic target in elderly patients.

Key words: Elderly, interleukin-33, periodontal disease

INTRODUCTION

Natural cellular senescence over an individual’s lifetime increases the risk for chronic conditions such as periodontal disease,[1,4] a spectrum of disorders in tissues that support and protect the dental organs, the development of which is associated with bacterial dental plaque.[5,6] Indeed, age is correlated with progressively worse periodontal health, as well as with a higher prevalence and severity of periodontal disease. Accordingly, periodontal disease disproportionately affects adults, of whom 10%–15% are estimated to suffer from severe periodontitis.[7,8]

Interactions between the immune system and oral bacteria are key factors that contribute to periodontal health and disease. In general, bacteria induce a local inflammatory reaction that activates the innate immune response through receptors on resident cells and leukocytes, including toll-like and nucleotide-binding oligomerization domain-type receptors, among others. Subsequently, activation of these cells elicits the production of pro-inflammatory cytokines and chemokines, as well as the recruitment of phagocytes and lymphocytes to the infected site.[9] Accordingly, changes in the innate and adaptive immune responses in old age may favor the development or manifestation of disease.[10] Indeed, the balance between innate immune response and acquired immune response (Th1/Th2), seems to be related to disease severity.[11] Ultimately, chronic periodontal disease activates or induces the secretion of several inflammatory proteins that promote bone resorption, resulting in loss of tooth matrix and support. Strikingly, the same inflammatory processes are implicated in other chronic diseases, including cardiovascular disease and diabetes.[12,14]

Given the prevalence and burden of periodontal disease in the elderly population, we surveyed...
the literature to investigate the relationship between inflammatory molecules and periodontal disease, with a view to identifying possible therapeutic targets.

**MATERIALS AND METHODS**

**Study design**

The literature was retrospectively and systematically surveyed for a subsequent meta-analysis. The selection, evaluation, disposition, and synthesis of the data were consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[16]

**Data extraction and inclusion/exclusion criteria**

We searched the North American database PubMed in three separate passes. In the first pass, the search was limited to the terms “pro-inflammatory protein,” “periodontal disease,” and “elderly.” For the second pass, we searched for “IL-33,” “periodontal disease,” and “elderly.” Finally, “IL-33” and “periodontal disease” were used as search terms for the third pass. The first and third passes were also restricted to the five years prior to October 20, 2016. Reviews and irrelevant studies were excluded. In addition, we excluded studies identified in the third pass that investigated the relationship between interleukin (IL)-33 and periodontal disease in the context of other diseases. Ultimately, authors, objectives, model, and conclusions were extracted from the included studies.

**Statistical analyses**

Data were reported as percentages and were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A nonparametric univariate Kruskal–Wallis test was used to compare independent groups with non-Gaussian distribution. Associations were evaluated by linear regression and Spearman’s test. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

The increased expression of immune mediators triggers chronic periodontal disease in elderly individuals. Thus, we first searched for publications using the terms “pro-inflammatory protein”, “periodontal disease,” and “elderly.” Surprisingly, of 35 publications identified from the last 5 years, only two investigated pro-inflammatory proteins and periodontal disease in the elderly [Flowchart 1a]. On the other hand, after a preliminary review of inflammatory signaling we found that periodontal disease was more strongly associated with molecules of the IL-1 family, especially the cytokine IL-33. Hence, we searched the database again for “IL-33,” “periodontal disease,” and “elderly,” but found only five studies investigating periodontal disease and IL-33, but not in the elderly [Flowchart 1b]. In the absence of articles directly associating IL-33 with the elderly, we expanded the search and used “IL-33” and “periodontal disease” only, and thereby...
identified 14 studies in the last 5 years, of which five were considered to be relevant [Flowchart 1c].

The two relevant studies identified in the first search [Flowchart 1a] were published in 2014 and 2016. In the former, fibroblasts from senescent human patients were found to be more vulnerable than fibroblasts from young patients to in vitro infection with *Fusobacterium nucleatum*. Infection also boosted caveolin-1 expression, suggesting that this molecule may be a key mediator of the host response. In the latter study, microwave treatment was found to modulate the expression of Ki67, a protein that suppresses p53 and TNF (Tumor necrosis factor)-α in the oral epithelium of young, middle-aged, and elderly individuals with chronic periodontitis [Table 1].

Of the five publications identified in the second survey, two each were published in 2012 and 2014, and one was published in 2015 [Table 2]. In one study published in 2012, IL-33 was found to be inadequate as a marker to differentiate between individuals with or without chronic periodontal disease. In the other study from 2012, no correlation was observed between the host genome and susceptibility to periodontal pathogens, although evidence suggestive of an association was found for 13 loci, including IL-33. Subsequently, a 2014 survey of inflammatory proteins in the crevicular fluid at different clinical stages of periodontal disease found that only IFN (Interferon)-γ levels were predictive. The other 2014 study reported that increased expression of IL-6 and IL-33 was associated with obstructive sleep apnea syndrome, indicating a potential relationship with periodontal disease. Finally, a notable 2015 study of cytokines in crevicular fluid showed that IL-36β was abundantly expressed in patients with aggressive periodontitis [Table 2].

After noting a potential relationship between IL-33 and periodontal disease, albeit not necessarily with the elderly, we assessed whether this relationship exists independent of potential relationships between IL-33 and other diseases [Table 3]. In this survey, we found five studies, of which 60% were published in 2016, 20% in 2014, and 20% in 2015. In the study published in 2014, IL-33 was found to correlate with TNF-α expression and with periodontal disease, apparently as a result of Th2 stimulation, as well as protective, anti-inflammatory, and repair processes. In one study published in 2015, increased expression of IL-33 and RANKL was demonstrated to be involved in the pathogenesis of periodontitis. The following year, the role of elevated IL-33 in periodontitis was again noted in patients. Remarkably, administration of IL-33 enhanced bone resorption, an effect potentiated by RANKL-dependent *Porphyromonas gingivalis*. In addition, in the same year, this pathogen was demonstrated to boost cytoplasmic IL-33 production as periodontal disease progressed. The increase in IL-33 was detected in the crevicular fluid, saliva, and the plasma of patients with chronic periodontitis, significantly highlighting the possible role of IL-33 in the pathogenesis of chronic periodontitis [Table 3].

We note that our analysis clearly suggests a rising interest within the research community, in the relationship between IL-33 and chronic periodontal disease in the recent years, with an average of approximately 2.4 publications/year. However,

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Table 1: Publications listed in PubMed from the last 5 years suggesting a correlation between pro-inflammatory proteins and periodontal disease in the elderly

| Author                  | Objectives                                                                 | Model                                      | Conclusion                                                                 |
|-------------------------|----------------------------------------------------------------------------|--------------------------------------------|---------------------------------------------------------------------------|
| Ahn et al. [19]          | Investigate mechanisms used by *F. nucleatum* to infect gingival fibroblasts | Young and senescent human gingival fibroblasts in vitro | Increased infection of senescent fibroblasts                              |
| Ianova et al. [20]       | Evaluate the effect of microwaves (radio and infrared) on the expression of pro-inflammatory cytokines and molecular markers of cell renewal (Ki67, p53) | Patients with chronic periodontitis         | Microwaves stimulate Ki67 and suppress p53 and TNF-α in the buccal epithelium of young, middle-aged, and elderly individuals |

TNF – Tumor necrosis factor; *F. nucleatum* – *Fusobacterium nucleatum*

Table 2: Publications listed in PubMed suggesting a correlation between interleukin-33 and periodontal disease in the elderly

| Author                  | Objectives                                                                 | Model                                      | Conclusion                                                                 |
|-------------------------|----------------------------------------------------------------------------|--------------------------------------------|---------------------------------------------------------------------------|
| Kurşunlu et al. [19]    | Evaluate IL-36β, IL-36γ, and IL-33 in crevicular fluid from patients with different forms of periodontal disease | Patients with or without generalized aggressive periodontitis, chronic periodontitis, and gingivitis | Elevated levels of IL-36β in patients with aggressive periodontitis        |
| Nizam et al. [19]       | Evaluate saliva PTX3, IL-1β, IL-6, IL-21, and IL-33 in patients with and without obstructive sleep apnea | Patients with and without moderate or severe obstructive sleep apnea | Moderate or severe obstructive sleep apnea syndrome boosts IL-6 and IL-33 levels, and a potential relationship may exist between the syndrome and periodontal disease |
| Papathanasiou et al. [20] | Evaluate IFN-γ, IL-4, IL-33, and lymphopoietin in crevicular fluid at different clinical stages of periodontal disease | Patients with chronic periodontitis or gingivitis due to bacterial plaque | Individuals with a caucasian phenotype, at risk of atherosclerosis, with or without periodontal disease at any stage of the disease |
| Divaris et al. [21]     | Analyze the association between the host genome and susceptibility to periodontal disease | Individuals with a caucasian phenotype, at risk of atherosclerosis, with or without periodontal disease | No significant association was observed, although evidence suggestive of association was noted in 13 loci, including IL-33 |
| Buduneli et al. [22]    | Investigate whether IL-33 levels in saliva, crevicular fluid, or plasma can identify individuals with chronic periodontal disease | Patients with chronic periodontitis or gingivitis due to bacterial plaque | No significant association was observed, although evidence suggestive of association was noted in 13 loci, including IL-33 |

IL – Interleukin; PTX3 – Pentraxin 3; IFN – Interferon
the frequency of publications did not increase linearly in the last 5 years, implying a non-Gaussian distribution [Figure 1].

Strikingly, our analysis varied significantly among human, mouse, and in vitro studies [Figure 2], which comprised 45%, 33%, and 22% of all studies, respectively [Figure 2a]. In particular, increased IL-33 was associated with chronic periodontal disease in 100% of the experiments in vitro and in mice, but only in 50% of the studies in humans [Figure 2b].

**DISCUSSION**

Since senescence contributes to the dysregulation of the immune system in the oral cavity, it is considered a risk factor for periodontal and other chronic systemic diseases, which are indeed more prevalent in the elderly. Accordingly, surveys to investigate the affected immune molecules will not only illuminate the pathophysiology of these diseases, but also identify new therapeutic targets. Surprisingly, we found only a small number of studies addressing this issue published at an irregular pace over the last 5 years. In addition, studies investigating the potential relationship between periodontal disease and increased expression of the pro-inflammatory protein IL-33 were more frequently conducted in mice in vitro rather than in humans.

In a clinical study of 42 patients at different clinical stages and with severity of periodontal disease, the levels of INF-γ in the crevicular fluid were found to be dependent on clinical stage. Remarkably, IL-33 was undetectable in these patients.

![Figure 1: Publication output in the last 5 years. Publications were identified in PubMed based on three distinct sets of search terms. One set consisted of search terms “proinflammatory protein,” “periodontal disease,” and “elderly.” A second set consisted of “IL-33,” “periodontal disease,” and “elderly.” The third set included “IL-33” and “periodontal disease” (P = 0.68 by Spearman’s test).](image1)

![Figure 2: Association between experimental model, periodontal disease, and interleukin-33. (a) Distribution of study models and (b) correlation between increased interleukin-33 and periodontal disease in different study models (P > 0.05 by Kruskal–Wallis test).](image2)

**Table 3: Publications listed in PubMed suggesting a relationship between interleukin-33 and periodontal disease**

| Author            | Objectives                                                                 | Model                                      | Conclusion                                                                                   |
|-------------------|-----------------------------------------------------------------------------|--------------------------------------------|---------------------------------------------------------------------------------------------|
| Sağlam et al. [23]| Compare IL-33 levels in the crevicular fluid, saliva, and plasma of patients with or without chronic periodontal disease | Human nonsmokers with or without chronic periodontitis and gingivitis | IL-33 may play a role in disease pathogenesis                                                |
| Tada et al. [24]  | Determine the role of *P. gingivalis* in inducing IL-33 as periodontal disease progresses | Gingival tissues collected from individuals with and without chronic periodontitis | *P. gingivalis* increases the expression of cytoplasmic IL-33 in human gingival epithelial cells in vitro |
| Malcolm et al. [25]| Investigate the role of IL-33 in periodontitis                              | Tissues from patients with or without chronic periodontitis; mice with experimentally induced periodontitis | *P. gingivalis* increases the expression of cytoplasmic IL-33 in human gingival epithelial cells in vitro |
| Köseoğlu et al. [26]| Investigate IL-33, RANKL, and osteoprotegerin in healthy periodontal tissue and in tissue with experimentally induced periodontitis | Wistar rats with periodontal disease induced by ligation | Increased IL-33 and RANKL may be associated with disease pathogenesis                        |
| Beklen and Tsaous Memet [27]| Investigate IL-33 levels in response to TNF-α                                        | Human periodontal tissues and gingival fibroblasts | TNF-α boosts IL-33 as a result of Th2 stimulation, protective, anti-inflammatory, and repair processes |

IL – Interleukin; TNF – Tumor necrosis factor; *P. gingivalis* – *Porphyromonas gingivalis*; RANKL – Receptor activator of nuclear factor kappa B ligand

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pathogens. However, evidence of an association was noted for 13 loci that regulate homeostasis and inflammation, including KCNK1, FBXO38, UHRF2, IL-33, RUNX2, TRPS1, CAMTA1, and VAMP3.[24,25] In contrast, a different survey suggested that crevicular fluid was elevated in individuals with gingivitis and chronic periodontitis compared to unaffected individuals, and that IL-33 levels in crevicular fluid were significantly lower in patients with chronic periodontitis than in patients with gingivitis and patients without periodontal disease.[23] Similarly, in another study, IL-33 activity was found to be associated with RANKL-dependent bone resorption, and thus with chronic periodontal disease.[26]

Taken together, these data suggest that variable genetic expression in patients infected with periodontal pathogens, as well as environmental factors, may elicit different infection control and repair pathways. Accordingly, the association between IL-33 expression and disease appears to depend on the infecting pathogen. In particular, P. gingivalis was shown to increase bone resorption and cytoplasmic IL-33 in human gingival epithelial cells.[24,25] Thus, while we noted some discrepancies, our survey generally supports a role for IL-33, presumably acting via the ST2 receptor, in the pathogenesis of chronic periodontal disease.[26,29]

**CONCLUSION**

The data reinforce the notion that inflammatory processes are involved in the development of chronic periodontal disease in the elderly. In particular, the data suggest a role of IL-33 in disease pathogenesis, also dependent on genetic and environmental factors. Thus, it is likely that IL-33 is a central mediator of periodontal disease and a possible therapeutic target.

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**Conflicts of interest**

There are no conflicts of interest.

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