SPECIALIZED PRO-RESOLVING MEDIATORS: RESOLVING INFLAMMATION AND RESTORING BONE

An Undergraduate Research Scholars Thesis

by

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Specialized Pro-Resolving Mediators: Resolving Inflammation and Restoring Bone

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Periodontitis is a chronic inflammatory disease characterized by gingival inflammation and loss of periodontal ligament attachment, which if severe enough can lead to the irreversible destruction of surrounding alveolar bone [1]. In chronic inflammation, hosts are in a prolonged state of dysbiosis characterized by more proinflammatory cells and cytokines. This results in a lack of their anti-inflammatory counterparts, stopping the ability of the host periodontium from resolving naturally. Under normal circumstances, inflammation is resolved, and the immune system is returned to baseline through the action of endogenous lipid-derived molecules called specialized pro-resolving mediators (SPMs). ***Specialized pro-resolving mediators*** (SPMs) are an

endogenous class of fatty acid-derived molecules that function to return the immune system to homeostasis. Recent pre-clinical studies showed that administered SPMs successfully resolved inflammation in rabbit and swine models of periodontitis. Phase I clinical trials using the lipoxin class of SPM in a mouth rinse demonstrated that SPMs were well tolerated by patients, and post-hoc efficacy analyses indicated that SPM therapy reduced gingival inflammation. Evidence also suggests that lipoxin administration reduced bleeding on probing and pocket depth in a subset of patients with advanced periodontitis. These studies collectively indicate that SPMs restore alveolar bone and regenerate periodontal ligament (PDL). This article will review the recent evidence demonstrating that SPM therapy resolves inflammation by inhibiting neutrophil chemotaxis and proinflammatory cytokine secretion. SPMs reduce bone resorption, restore alveolar bone, and regenerate PDL. Although further clinical studies are needed to evaluate the best SPM class and dosage, these data indicate that SPM therapy has the potential to transform the treatment of periodontitis.

DEDICATION

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NOMENCLATURE

SPM	Specialized pro-resolving mediators
PDL	Periodontal ligament
GI	Gastrointestinal
T-reg	T-regulatory cell
GF	Gingival fibroblast
IL	Interleukin
PDLF	Periodontal ligament fibroblast
Th	T-helper
DC	Dendritic cell
MHC	Major histocompatibility complex
APC	Antigen-presenting cell
NSAIDs	Non-steroidal anti-inflammatory drugs
COX	Cyclooxygenase

INTRODUCTION

Periodontal disease (periodontitis) is an inflammatory disease of the periodontium characterized by periodontal ligament loss and destruction of surrounding alveolar bone. Periodontitis has an estimated prevalence of 61.9% in the world population with progression to severe stages in 16.8% [1,2]. The tissue loss caused by severe periodontitis frequently results in loosened and lost teeth. Further, the sustained inflammatory state caused by this disease predisposes those it afflicts to systemic inflammatory conditions such as diabetes, arthritis, and inflammatory bowel disease. Thus, periodontitis is a major world-wide health problem.

Periodontitis begins as an infection of the gingiva induced by chronic exposure to biofilm in dental plaque [3,4]. Nonspecific accumulation of plaque and the lack of removal, commonly exacerbated by poor oral hygiene practices, leads to an inflammatory response within the gingival tissues. Immune cells migrate to the site of infection to kill and remove pathogens. However, when these cells fail to clear pathogens or infection persists from continued microbial exposure, tissue repair and return to homeostasis is prevented. Instead, inflammation becomes chronic [5]. Chronic inflammation induces the adaptive immune response, which further increases recruitment and activation of proinflammatory cell types [6,7]. These cells secrete cytotoxic enzymes and substances originally intended to destroy the invading pathogens, but their sustained presence causes increased tissue damage. Thus, the host response, not bacterial infection, causes the long-term destruction seen in periodontal disease.

With disease progression, the persistently elevated levels of inflammatory cytokines released by immune cells in the periodontium stimulate monocyte-derived osteoclasts to reabsorb bone, while osteoblast-mediated bone production is suppressed. This change in

homeostatic balance is caused by persistently elevated levels of immune cells in the periodontium [8,9]. As gingival tissue is degraded, followed by the periodontal ligament, and then bone resorption, pockets form and deepen. The anaerobic environment within these pockets favors a shift further toward gram-negative bacteria, especially *P. gingivalis, T. denticola, and T. forsythia*. These "red complex" bacteria are the hallmark pathogens of periodontal disease. The proliferation of these bacteria contributes to dysbiosis that amplifies the inflammatory response, further enriching the environment with tissue breakdown products that enhance their growth[10]. Thus, a feed-forward cycle of infection, inflammation, and destruction spins out of control and results in irreversible tissue loss.

Under normal circumstances inflammation is resolved and the immune system is returned to baseline through the action of endogenous, lipid-derived molecules, called specialized pro-resolving mediators (SPMs). These arachidonate-derived molecules are produced by resident tissue cells and immune cells, bind to their receptors on immune cells, and shift their phenotype to a post-inflammatory state conducive to tissue healing. The local pathological environment in chronically inflamed periodontal tissue suppresses normal SPM synthesis, allowing the disease to continue [11,12].

Current treatments for periodontitis are aimed primarily at physically removing the biofilm responsible for initiating infection and inhibiting its growth with antibiotics. Scaling and root planing is the first line of treatment to remove pathogenic organisms and other foreign debris from the oral tissue. Antibiotic medications kill pathogenic organisms. Together, these methods aim to reduce the bacterial load and minimize inflammatory stimulus [13-15]. Anti-inflammatory medications are sometimes used to block the cyclooxygenase enzymes that synthesize prostaglandins and alleviate initiation of inflammation with eventual resolution [16].

More invasive surgical periodontal therapy is sometimes used to recontour irregular bone patterns caused by resorption as a result of advanced disease [17,18]. All these therapies attempt to create an environment more conducive to resolve inflammation. However, none of them directly influence the inflammatory mechanisms underway once activated. Nor do they act upon or take advantage of the body's innate resolution mechanism.

Understanding the pathogenesis of periodontitis as an inflammatory disease rather than an infectious disease offers the potential to change the treatment paradigm. Recent studies have demonstrated that application of exogenous SPMs to facilitate the natural resolution of inflammation is effective in several chronic inflammatory diseases, and this strategy is now being explored in periodontitis [11,19].

This work will review the pathogenesis of periodontitis, including its underlying inflammatory mechanisms and their natural resolution by SPMs. It will then describe the current data on SPM usage in inflammatory disease treatment, and its application to the treatment of periodontitis as an alternative to conventional therapies.

1. INFLAMMATORY RESPONSE

Chronic inflammatory disease is a pathological maintenance of the host immune response to infection or other inflammatory stimuli. The sustained host response is responsible for the tissue damage and disease progression [20,21]. Current efforts at treating chronic inflammatory disease, including periodontal disease, are thus aimed at understanding, and targeting the cellular mechanisms underlying that host immune response to infection. This section will review current knowledge of those mechanisms in the context of periodontal disease.

The immune system functions to prevent or eliminate infection [3]. The innate immune system is the body's initial defense, designed to attack invading organisms upon first contact. Neutrophils, eosinophils, basophils, mast cells, monocytes/macrophages, and dendritic cells constitute this group. Their main function is to respond quickly to a microbial infection and engulf or digest the invaders while activating the adaptive immune response that follows. That response includes the participation of various subtypes of T and B lymphocytes that are synthesized to recognize specific epitopes on invading organisms and thus more efficiently identify and destroy them than innate mechanisms alone.

Periodontitis is a disease of dysbiosis resulting from a shift in the types of invading organisms away from normal oral microbiota to more pathogenic species. Initial invasion of soft gingival tissues by chronic exposure to the flora in plaque activates the innate immune response, causing the digestion of gingival collagen and the formation of pockets. As these pockets deepen, a localized region of edematous tissue is surrounded by nutrient-filled exudate forms. The anaerobic environment created in these pockets favors the multiplication of gram-negative bacterial species as opposed to normal gram-positive aerobic ones. The initial shift causes the

predominance of what is termed the "orange complex," composed of *Prevotella intermedia*, *Prevotella nigrescens*, *Prevotella micros*, and *Fusobacterium nucleatum*. As inflammation continues in response to these pathogenic species, tissue damage extends to the periodontal ligament, and pockets deepen. The orange complex species give way to the "red complex" bacteria *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), and *Porphyromonas gingivalis* (*P. gingivalis*) classically associated with advanced periodontitis [22,23]. This protected microenvironment tailored to nurture these pathogenic species provides the setting for the elevated and unrelenting inflammatory host response that ultimately results in permanent tissue loss.

Figure 1 diagrams the actions of the red complex in the presence of an imbalanced host defense and persistent unresolved inflammation in the periodontium. Beginning at the site of disease, Mast cells and resident tissue macrophages recognize microbial structures and factors released by damaged tissue [24,25]. These cells release histamines, prostaglandins, and chemokines with the collective effect of increasing vascular permeability and emigration of leukocytes from the capillaries to the site of infection.

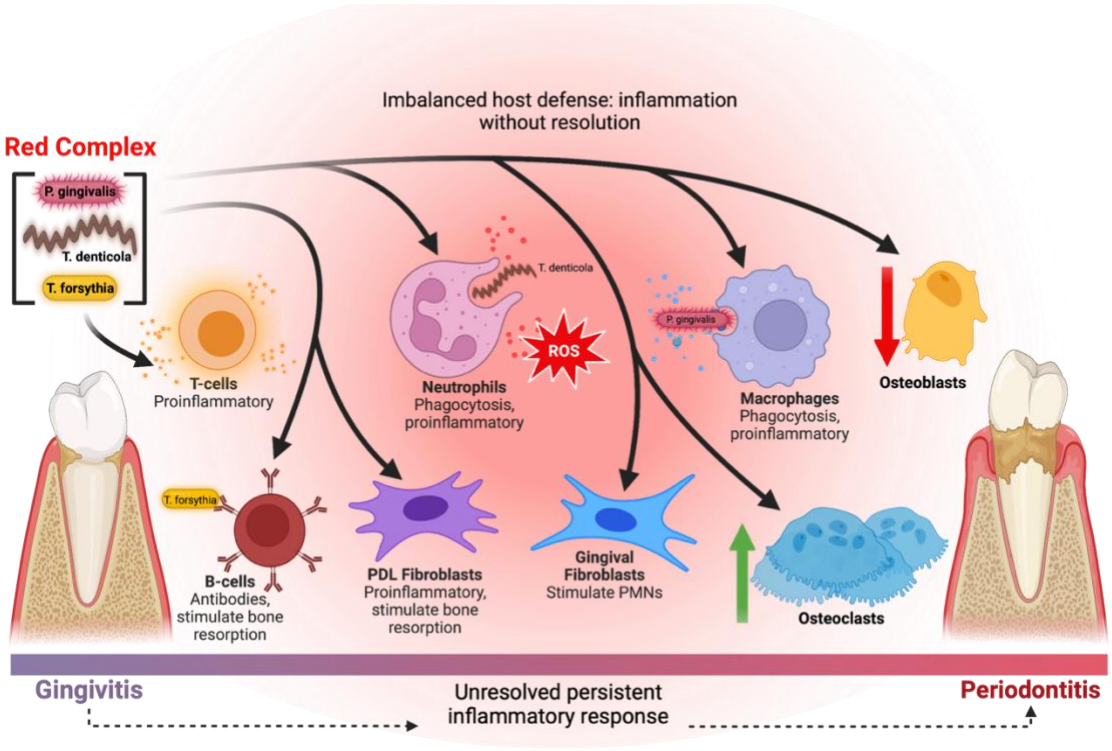

Figure 1. Imbalanced host defense and the effects of unresolved inflammation*. Three oral pathogens (Red Complex) that induce the inflammatory response in host cells that leads to gingivitis and periodontitis. Cytokines induced by this response increase osteoclast activity and decrease osteoblast activity leading to the destruction of alveolar bone and periodontal ligament attachment. Created with Biorender.*

Neutrophils are the first responders of the immune system. These phagocytic cells extravasate from the blood stream and become activated by the local signals to engulf microbial pathogens while also producing reactive oxygen species (ROS) and a panel of proteolytic enzymes. These substances are non-specific in nature. ROS oxidize and destroy bacterial structures and host tissue alike, while the enzymes, such as elastase, digest both pathogens and host tissue. Evolution has determined that this limited and localized destruction of host tissue is a suitable price to pay for the elimination of the invaders, and normally such damaged tissue is restored in the subsequent healing phase [26,27]. However, the longer that inflammation persists, the more activated neutrophils are recruited, and the more tissue is damaged by the substances they secrete. Additionally, as more are recruited to a specific site, the toxic lysis products from

expended neutrophils accumulate, and the delay in removal of these waste products directly promotes tissue damage. As inflammation extends into the chronic phase without transition to resolution, neutrophils increasingly do more harm than good.

Monocyte-derived macrophages are the second type of phagocytic cell to populate the site of infection. They play several roles in initiation, potentiation, and resolution of inflammation, depending on context [28]. For this discussion's purposes, macrophages exist at the injury site as pro-inflammatory M1 cells or as anti-inflammatory/pro-resolution M2 cells. M1 macrophages play a vital role in localized vasodilation and recruitment of neutrophils, accelerating the innate response through their secretion of proinflammatory cytokines and chemokines. At the same time, they function as antigen-presenting cells (APCs) by presenting epitopes from phagocytosed pathogens on their surface to activate the adaptive immune response. And finally, M2 macrophages enable the resolution phase, both by secreting anti-inflammatory cytokines and by functioning as the primary "clean-up" cell during the return to homeostasis. As neutrophils undergo programmed cell death (apoptosis), resolution-phase macrophages remove the leftover cytotoxic cellular debris to make way for tissue healing. Macrophage phenotype switching depends strongly on local cytokine production by T-cells of the adaptive immune system and on production of SPMs by resident tissue cells [29].

Dendritic cells (DCs) are also APCs. In fact, they are the primary APCs in the activation of T and B cells. Immature DCs patrol the oral tissues and phagocytose pathogenic fragments. From there, they travel to the lymph nodes, where they mature and present these antigens with major histocompatibility complex (MHC) class II proteins to T-helper (Th) cells. The maturity stage of DCs influences their capacity to present antigens, and thus their preference for activation

of proinflammatory or anti-inflammatory Th types (detailed below) [30-32]. This means that control of DC maturity is a major influence in the system's decision to maintain or resolve inflammation.

Cells unique to periodontal tissues involved in innate immunity include periodontal ligament fibroblasts (PDLFs) and gingival fibroblasts (GFs). PDLFs function to attach teeth to surrounding bone via the periodontal ligament. However, in the presence of pathogenic bacteria and proinflammatory products, they also release cytokines that are inflammatory and promote osteoclast activity. GFs are highly reactive to foreign pathogens. In the presence of foreign invaders, GFs are stimulated to release proinflammatory molecules and increase the reactivity of neutrophils [33].

B cells serve to differentiate into antibody-producing plasma cells and memory cells [34]. Plasma cells produce antibodies that bind to pathogens to mark them for cell death. Studies have demonstrated that B cells sensitized to periodontal pathogens also have the capability to stimulate osteoclast activity and thus may play a significant role in periodontitis-induced bone resorption [33,35].

T lymphocytes are major determinants of inflammatory progression in the periodontium [36]. T cells mature in lymphatic tissues and differentiate primarily into CD4+ T-helper (Th) or CD8+ cytotoxic cells. CD8+ cells are adaptive MHC I-displaying cells that bind epitopes of specific pathogens to "dock" with them and release enzymes that kill them. Th cells, as their name implies, activate other immune cells to help facilitate their actions in cell-based or humoral immunity. We will focus this discussion on four specific types of Th cells that play critical roles in periodontal inflammation: Th1, Th2, Th17, and Treg. The complex interplay between each of these cell types and their connections through the cytokine network is diagrammed in Figure 2.

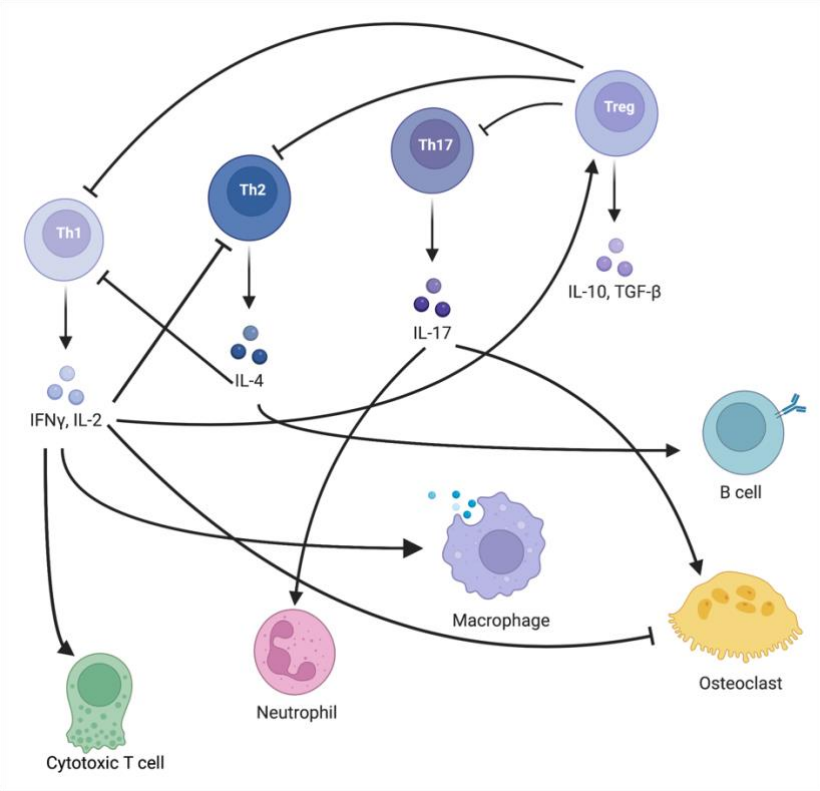

Figure 2. Simplified diagram of the cytokine network connecting Th cells with inflammatory cells. *Th cells develop from naïve CD4+ cells, while cytotoxic T cells derive from the CD8+ population. See text for details. Created with Biorender.*

Th1 cells act to promote cell-mediated immunity and regulate autoimmunity while Th2 cells promote humoral immunity. Interferon-gamma (IFN-γ), the major cytokine produced by Th1 cells, activates macrophages and cytotoxic T cells, while Interleukin-4 (IL-4), the major product of Th2 cells, potentiates B cell differentiation [37]. Th1 and Th2 cells regulate each other's numbers [38]. IL-2 produced by Th1 cells inhibits CD4+ progenitor differentiation into Th2 cells, while Th2-produced IL-4 inhibits Th1 differentiation. Despite their role as macrophage activators, Th1 cells appear to have a protective function in periodontitis in that they suppress osteoclast differentiation [39]. Th1 cells do so by switching monocytes from an osteoclastic to a macrophage lineage, even though they are often viewed as pro-inflammatory in their cytokine

profile. The role of Th2 cells in tissue destruction is less clear. In some experimental models, these cells are protective, while in others, they associate with tissue damage, suggesting that their role is context dependent [33].

Th17 cells are so named for their primary cytokine product, IL-17, and they play a role primarily in microbial immunity. The cells promote osteoclastogenesis, and thus bone destruction, as well as neutrophil proliferation and activation. Thus, their overall effect is considered pro-inflammatory and destructive in the periodontium.

T regulatory (Treg) cells inhibit expansion of the other Th types and suppress osteoclast differentiation and macrophage activation [40]. Thus, they suppress inflammation and promote resolution [41-43]. Interestingly, one of their primary secreted factors, transforming growth factor-beta, has an anti-inflammatory effect on Th17 cells. Tregs are present in limited numbers in diseased periodontium. However, their numbers increase during the resolution phase, indicating their importance in the return to homeostasis. Treg multiplication is spurred by the IL-2 secreted from Th1 cells, thus enabling the beginnings of resolution even as inflammation ramps up.

2. INFLAMMATORY RESOLUTION AND THE ROLE OF SPMS

Return to tissue homeostasis is not merely a passive cessation of inflammation. Resolution is an active process, enacted by phenotype switching of immune cells and mediated by an underlying shift in cytokine expression. Thus, the normal inflammatory process is a highly orchestrated sequence of events in which inflammation peaks, recedes, then gives way to resolution, and finally tissue healing and homeostasis. Chronic inflammation is not simply the maintenance of the proinflammatory state, but the hindrance of resolution.

The seeds of resolution germinate at the height of inflammation. The proinflammatory prostaglandins and cytokines produced by mast cells, tissue macrophages, and activated neutrophils activate transcription of genes in these cells that switch arachidonic acid from prostaglandin synthesis over to production of specialized pro-resolving mediators (SPMs) [27].

Figure 3 illustrates the synthesis of each class of SPM from dietary polyunsaturated fatty acids. Omega-6 fatty acids further break down into arachidonic acid (AA) which reduce to lipoxins. Lipoxins activate antimicrobial defense mechanisms by promoting the release of anti-inflammatory cytokines and inhibiting inflammatory cells and their cytokines as well as stimulating the uptake of apoptotic neutrophils.[11] Omega-3 fatty acids break down into two molecules: Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). EPA can be reduced to the resolvins E series SPMs while DHA can be broken down into the resolvins D series, protectins, and maresins.[44-45].

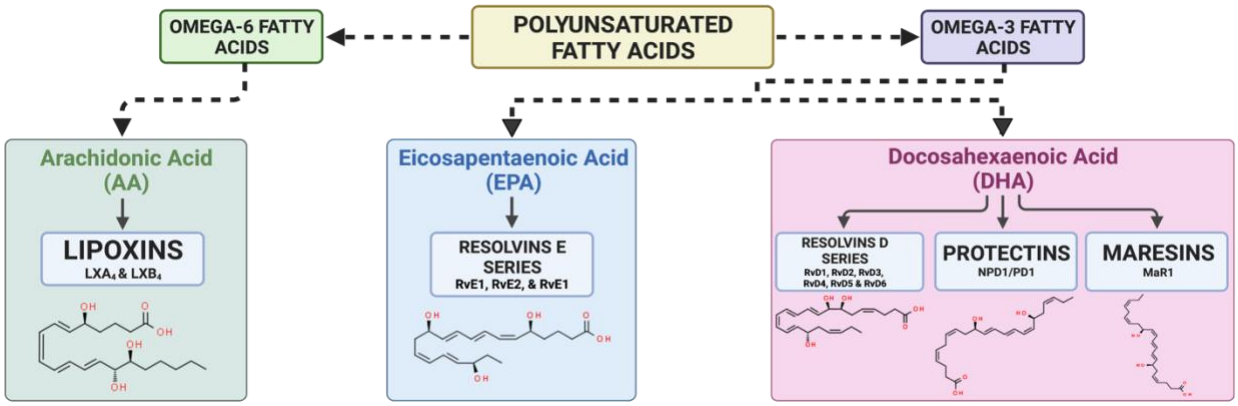

Figure 3. SPMs classes are derived from polyunsaturated fatty acids. *Lipoxins derived from Arachidonic acid. Resolvins E series are derived from Eicosapentaenoic acid. Resolvins D series, Protectins and Maresins are derived from Docosahexaenoic acid. Adapted from J Periodontal. 2020;91 (Suppl 1):S19-S25. doi:10.1002/JPER.20-0088.*

Lipid mediator class switching from pro-inflammatory eicosanoid synthesis to SPMs happens in neutrophils, macrophages, and Treg cells [46]. The SPMs bind to their G-protein coupled receptors and induce neutrophils to undergo programmed cell death, efferocytosis of dying neutrophils by macrophages that switch from the pro-inflammatory M1 to the pro-resolving M2 phenotype, and a shift of T cells from Th1 and Th2 lineage to a Treg commitment [12,47]. The result of this realignment toward resolution is that neutrophils cease their extravasation and proliferation and undergo apoptosis, macrophages clean up the fragments of dying neutrophils instead of releasing pro-inflammatory cytokines, and T cells reset adaptive immunity to long term tolerance and homeostasis (Figure 4). An important feature of resolution is that it does not leave the immune response helpless against renewed infection by pathogenic organisms, but rather resets the adaptive immune response to program memory B and T cells for quick response to later infection. SPMs inhibit the conversion of naïve T cells to pro-inflammatory Th cell types and shift them to the Treg lineage. The increase in the Treg cell population, also encouraged by Th1-produced IL-2, further inhibits inflammatory IL-17 cytokine production from

Th17 cells and promotes a return to wound healing and homeostasis. Treg cells also promote osteoblast activity to grow new bone and inhibit osteoclast resorption [41].

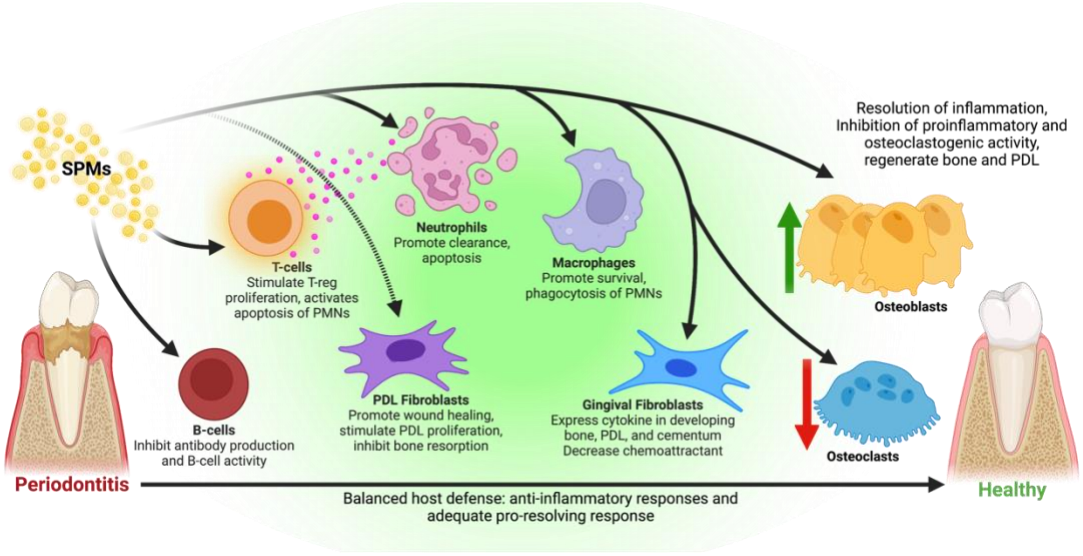

Figure 4. SPMs resolve the inflammatory response. *SPMs inhibit lymphocyte activity, promote neutrophil apoptosis and phagocytosis by macrophages, while also shifting osteoblast/osteoclast activity balance toward bone formation to reestablish normal bone homeostasis. Created with Biorender.*

The presence of active resolution pathways presents targets for manipulation in resolution-mediated therapies, rather than just inhibiting the pro-inflammatory stimulus. Further, because resolution stimulates tissue healing, pharmacological targeting of resolution pathways offers the potential for tissue restoration rather than just conservation of remaining tissue.

3. CURRENT PERIODONTAL THERAPY OPTIONS

Standard of care for periodontitis consists largely of non-surgical and surgical techniques to remove the pathogenic biofilm and with it the inflammatory stimulus in the hopes that tissue destruction will slow and perhaps cease [3,48]. The objective is to then maintain a healthy periodontium by a combination of professional care and patient self-care. The current first line treatment of chronic periodontitis is nonsurgical periodontal debridement (NSPD) also known as scaling and root planing (SRP). SRP involves the use of various hand instruments and ultra-sonic scalers.

Hand instrumentation requires the clinician to exert moderate to heavy lateral pressure to remove calculus. This force fatigues the operator over time and can potentially damage tooth structure. With incorrect angulation and instrumentation, patient tooth structure can be damaged causing sensitivity and increasing the risk of developing carious lesions.

Thus, SRP is commonly accomplished with ultrasonic scalers, such as the Cavitron and Piezo-electric [13]. These instruments decrease operator fatigue and increase efficiency of calculus and biofilm removal. Through the mechanism of a longitudinal or elliptical movement, calculus, and biofilm are easily removed with the correct technique. The variance in the reliability of different clinicians determines the success of this method. By disrupting bacterial-produced biofilms and removing hard deposits, the source of the inflammatory stimulus is removed mechanically. However, some more virulent bacterial strains have adhesive properties that allow them to avoid mechanical removal. In addition, if the bacteria are not reached through SRP due to clinician accessibility or pocket depths greater than 5 mm, those pathogens remain in diseased tissue and require more invasive treatment.

If there is significant destruction past the ability of an instrument alone to fully debride the teeth, surgical debridement is required. Given the state of severely diseased tissue and the response of cells involved in bone destruction, the periodontium becomes disfigured. Bone loss in a diseased pattern leaves alveolar bone serrated, which in return creates surfaces favorable for bacterial preservation and expansion and, subsequently, loss of dentition. To avoid further infection and tooth loss, surgical treatments are required to treat periodontitis to restore a symbiotic environment. These treatments include open flap debridement, osseous resective surgery, and periodontal regeneration.[17,49]

In periodontal flap surgery, incisions are made in the gingiva to allow for separation of the epithelial tissues from the roots and alveolar bone. This access allows clinicians to remove calculus and biofilm past the 5 mm mark instead of relying solely on tactile sensitivity, as during the SRP procedure. Osseous resective surgery uses a combination of an ostectomy and osteoplasty. This functions to restore the alveolar bone to a pre-disease contour and thus the overlying gingiva.

Periodontal regeneration therapy includes various combinations of membranes, bone grafts, a combination of bone graft and membrane, and biologics. Biologics include enamel matrix derivatives, platelets preparations, and growth factors that function to encourage growth of bone and the overlying periodontium [49,50]

Antibiotics and anti-inflammatory drugs are also used as adjuvants in the treatment of periodontitis. Antibiotics are locally and systemically applied to remove pathogenic microorganisms that remain after physical cleaning. The infectious agents inhabit gingival pockets where the patient's normal oral hygiene practices have trouble reaching. Antibiotics are typically indicated for chronic or aggressive periodontitis with ongoing tissue damage [51]. Typical

antibiotic selections are tetracycline and derivatives, Augmentin, and metronidazole. However, long-term antibiotic use risks the development of antibiotic resistance and impaired host defense against other organisms. Further, pH differences in the anaerobic microenvironment may inhibit antibiotic effectiveness. Thus, antibiotic treatment is only a supplementary treatment for periodontal disease. It cannot address the inflammatory cascade, only assist in the temporary reduction of the microbial stimulus.

Non-steroidal anti-inflammatories (NSAIDs) stop initiation of the inflammatory response by blocking the cyclooxygenase (COX) enzymes that synthesize prostaglandins from arachidonic acid [16,52,53]. Thus, NSAIDS inhibit vascular permeability, neutrophil activation, pro-inflammatory interleukin secretion, and Th cell differentiation. However, anti-inflammatory medications are not safe to use chronically, as they can result in detrimental effects to the cardiovascular system, liver, kidneys, and gastrointestinal tract [53,54]. Further, they only inhibit initiation of an inflammatory process that has already begun, and by inhibiting COX enzymes, they suppress the lipid mediator class switching that reorients arachidonic acid toward lipoxin synthesis. Therefore, while NSAIDS slow inflammation, they also stunt resolution and prevent return to homeostasis.

In summary, the current standard of therapy for periodontitis is to remove the pathogenic microbial stimulus and stifle the initiation of inflammation. Current therapies do not subdue the inflammatory cascade once it has proceeded to the self-sustaining chronic phase, nor do they allow for natural resolution that leads to healing and homeostasis.

4. SPECIALIZED PRO-RESOLVING MEDIATORS IN PERIODONTAL THERAPY

The discovery of SPMs and their cellular receptors present opportunities to activate the endogenous resolution programs that are inhibited in chronic inflammation. Stabilized analogs of these lipid mediators have been shown as effective agonists of resolution pathways, able to tip the balance toward homeostasis in chronic inflammatory diseases. As endogenously produced molecules, SPMs do not suppress the immune system which means treatment is well tolerated and can be maintained over longer periods of time in comparison to anti-inflammatory medications.

SPM therapy is currently used to treat patients with arthritis and chronic pain and is being evaluated for the treatment of gastrointestinal (GI) inflammation, Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS), all of which display components of sustained inflammation [45,55-60]. Elevating the levels of resolvins in synovial fluid has proven to reduce the inflammation associated with arthritis, resulting in significant reduction in pain intensity [61]. Because SPMs do not suppress normal immunity and potentiate adaptive immunity, they have also shown potential for lowering antibiotic dosages and course of treatment necessary to treat infection, thus preventing antibiotic resistance [60].

The successful resolution of chronic inflammation by exogenous SPM application in other chronic diseases prompted pre-clinical investigation of their effectiveness in treating periodontal disease. The first of these studies used a rabbit model of induced periodontitis established through ligature and application of the red complex bacterium, *P. gingivalis*, for six weeks [62]. Rabbits prepared through ligature demonstrated significant alveolar bone loss. At the

initial six-week mark, bone loss in bacteria-induced rabbits averaged 30% compared to the ligature-only group. Rabbits were either treated with vehicle alone or Resolvin E1 (RvE1) for an additional six weeks. The authors of this study chose RvE1 because of previous evidence that eicosapentaenoic acid (EPA) containing lipids, metabolic precursors to resolvins (Figure 3), suppress inflammation in several human diseases [63,64]. Whereas animals treated with vehicle alone showed advancement of the disease as measured by increased bone loss and tooth mobility over this time, topical treatment with RvE1 three times a week for six weeks resulted not only in cessation of inflammation but also restoration of alveolar bone to near pre-disease levels. Tooth mobility in the resolvin treated group was also eliminated. Pocket depths in inflamed rabbits that averaged 4 mm before treatment were reduced to less than 1 mm by RvE1 treatment, while untreated pockets went on to deepen to over 7 mm. Restoration of damaged tissue is not seen in standard periodontal disease therapy. The fact that resolvin treatment not only halted disease but reversed its effects was a striking demonstration of the potential for resolution pharmacy in periodontal care.

In patients with aggressive periodontitis, neutrophils produce increased amounts of the lipoxin LXA4 compared to those without periodontal disease. However, the lipoxins produced were either too little in substantivity or incapable of binding to receptors [65]. This was proven by a failure of resolution and progression of disease. In order to further explore the potential for SPM treatment of human periodontal disease, Van Dyke and coworkers applied a lipoxin analog to a porcine model of periodontitis [66]. Unlike rodents, pigs can develop periodontitis in gingival defects without applying red complex bacteria, thus simulating the disease etiology in humans more closely. And, as in humans, tissues lost to disease are not restored. Porcine chronic periodontitis was treated with surgical debridement followed by membrane vesicle particles

containing the stable lipoxin analog BLXA4. These so-called nano-proresolving medicine (NPRM) particles alone had little effect after three months of treatment, while treatment with lipoxin-conjugated NPRM-BLXA4 particles resulted in significant regrowth of bone and periodontal ligament.

5. PHASE I CLINICAL TRIAL

The striking success of SPMs in not only arresting but reversing periodontal damage in animal models led to the first clinical trial of SPMs as treatment of chronic gingival inflammation. Phase I trials are aimed at assessing the safety of therapeutic treatments in patients, although data is collected for limited post-hoc assessment of efficacy that can be used to inform and justify further trials. This study focused on the use of a stable analog of lipoxin A4, denoted BLXA4, in inflammatory periodontal disease [56]. Treatment was provided in the form of an ethanol-based mouth rinse. The clinical trial allocated 127 subjects into three groups: a vehicle alone mouth rinse without lipoxin, a treatment with lipoxin, and a no treatment group. 50 subjects were part of a vehicle rinse group, 50 were given the BLXA4 rinse treatment, while the remaining 27 were assigned to use no rinse at all. Patients were enrolled based on positive diagnoses of gingival inflammation ranging from gingivitis to severe periodontitis. The trial reported no ill effects from the treatment, validating the safety of SPM treatment and paving the way for further efficacy trials. Further, the study showed after 14- and 28-day time points a statistically significant reduction in modified gingival index (MGI) of approximately 20% for the BLXA4 treatment group compared to vehicle rinse. The MGI uses a visual scale to access gingival health scoring from 0-4 (healthy-localized mild, generalized mild, moderate, and severe) by trained clinicians blinded to group assignment of the patients.

Another key clinical endpoint associated with gingival inflammation, bleeding on probing, was measured using a dichotomous measure, as 1=bleeding 0=no bleeding within 15 minutes following probing of the site. Change from baseline at Day 14 and Day 28 was

compared between groups. The $BLXA_4$ group demonstrated greater reduction in bleeding compared to vehicle group at both Day 14 and Day 28 (*$p<0.0001$; *$p=0.0053$, respectively).

As pocket depth is a primary measure of the severity of chronic periodontitis, change in probing depth was also measured in a subgroup of subjects exhibiting periodontal disease with pocket depths ≥ 6 mm at baseline (denoting serious progression of disease). There were 5 subjects in the BLXA4 group, 7 subjects in the placebo group, and 5 subjects in the no rinse group. Change over time revealed that periodontal pocket depth reduction (-1.23 ± 0.4 mm) with a 1.22 ± 0.6 mm clinical attachment gain was most pronounced in the $BLXA_4$ group compared to the placebo group (-0.71 ± 0.3 mm in PD with a CAL gain of 0.72 ± 0.3 mm). However, this change was not statistically significant, possibly due to both the variability starting pocket depth of the sample population and the small number of patients in each group.

Serum levels of all four classes of SPMs were increased by the oral lipoxin treatment compared to controls. This finding suggested that resolution of inflammation in the oral cavity may increase systemic protection against inflammatory stimuli.

The limited efficacy data from this first ever clinical trial of SPMs in periodontal disease paved the way for future patient studies on the full potential of SPM effects in oral health care. The data obtained from this phase I trial did not establish the ability of lipoxin treatment to restore alveolar bone and PDL as seen in animal models. However, sample sizes were meager, and variability was high in this subgroup. These factors may have obscured observation of a significant difference. Further, a longer course of treatment may possibly have yielded a statistically significant improvement. Overall results were encouraging, and serum levels of SPMs indicated that pro-resolution molecules of all types may be elevated by administration of

just one type of molecule. Additional studies should reveal which class or classes of SPM produce the best results in resolution of periodontitis and at what dosages and treatment courses.

CONCLUSION

Periodontitis is a disease of inflammation induced by periodontal pathogens. The host's inflammatory response is key in the progression and severity of disease.[1,2] Current treatments for SPMs include SRP, surgical periodontal therapy, antibiotics, and anti-inflammatory medications (NSAIDs).[49,52,53], but these treatment methods act only to reduce initiation of the inflammatory response. They do not facilitate its progression to healing and homeostasis. Rather, the inflammatory pathways remain locked in a cycle of tissue destruction.

SPMs show promising results as a new class of treatments known as "resolution pharmacy." Treatment with SPMs work with the immune response instead of suppressing it. Current studies on the use of SPMs have led to a new perspective on treatment of inflammatory diseases, including periodontitis. For the first time, therapies with lipoxins and resolvins have been shown to be effective in not only halting the progression of periodontitis but in reversing its tissue damage and restoring healthy periodontium.[56] Taken together, these studies represent a shift in the treatment paradigm away from fighting the immune response, to working with it.

The path toward SPM use as standard of care in periodontal disease still has several milestones to be reached. Which SPM classes yield the best results and in what combinations must be determined. Also, in question is whether SPMs should be used as preventatives to avoid gingival inflammation or reserved for the treatment of acute disease. Also, yet to be determined is whether SPM application is most effective when combined with clinician-performed treatments like SRP, or if it can be used alone to a significant effect.

SPMs are not the only target for resolution pharmacy. Inhibitors and activators of a host of interleukins, cytokines and chemokines involved in the switch from initiation of inflammation

to resolution remain to be explored [12]. Studies on these other pathways may eventually be combined with SPM therapy to even greater effect than the benefits now being seen with SPMs alone. Nevertheless, alone or in combination, specialized pro-resolving mediators represent a revolution in the treatment of periodontitis.

REFERENCES

1. Germen M, Baser U, Lacin CC, Firatli E, Issever H, Yalcin F. Periodontitis Prevalence, Severity, and Risk Factors: A Comparison of the AAP/CDC Case Definition and the EFP/AAP Classification. *Int J Environ Res Public Health*. Mar 26 2021;18(7)doi:10.3390/ijerph18073459

2. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. *Int J Health Sci (Qassim)*. Apr-Jun 2017;11(2):72-80.

3. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers*. Jun 22 2017;3:17038. doi:10.1038/nrdp.2017.38

4. Haffajee AD, Socransky SS. Microbiology of periodontal diseases: introduction. *Periodontol 2000*. 2005;38:9-12. doi:10.1111/j.1600-0757.2005.00112.x

5. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol 2000*. Feb 2014;64(1):57-80. doi:10.1111/prd.12002

6. Kinane DF, Demuth DR, Gorr SU, Hajishengallis GN, Martin MH. Human variability in innate immunity. *Periodontol 2000*. 2007;45:14-34. doi:10.1111/j.1600-0757.2007.00220.x

7. 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*. Dec 2012;27(6):409-19. doi:10.1111/j.2041-1014.2012.00663.x

8. Hienz SA, Paliwal S, Ivanovski S. Mechanisms of Bone Resorption in Periodontitis. *J Immunol Res*. 2015;2015:615486. doi:10.1155/2015/615486

9. Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B. Bacterially induced bone destruction: mechanisms and misconceptions. *Infect Immun*. Jul 1996;64(7):2371-80. doi:10.1128/iai.64.7.2371-2380.1996

10. Mohanty R, Asopa SJ, Joseph MD, et al. Red complex: Polymicrobial conglomerate in oral flora: A review. *J Family Med Prim Care*. Nov 2019;8(11):3480-3486. doi:10.4103/jfmpc.jfmpc_759_19

11. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature*. Jun 5 2014;510(7503):92-101. doi:10.1038/nature13479

12. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol*. Dec 2005;6(12):1191-7. doi:10.1038/ni1276

13. Oosterwaal PJ, Matee MI, Mikx FH, van 't Hof MA, Renggli HH. The effect of subgingival debridement with hand and ultrasonic instruments on the subgingival microflora. *J Clin Periodontol*. Oct 1987;14(9):528-33. doi:10.1111/j.1600-051x.1987.tb00995.x

14. Matia JI, Bissada NF, Maybury JE, Ricchetti P. Efficiency of scaling of the molar furcation area with and without surgical access. *Int J Periodontics Restorative Dent*. 1986;6(6):24-35.

15. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. *Ann Periodontol*. Dec 2003;8(1):115-81. doi:10.1902/annals.2003.8.1.115

16. Ren J, Fok MR, Zhang Y, Han B, Lin Y. The role of non-steroidal anti-inflammatory drugs as adjuncts to periodontal treatment and in periodontal regeneration. *Journal of Translational Medicine*. 2023/02/25 2023;21(1):149. doi:10.1186/s12967-023-03990-2

17. Wang HL, Greenwell H. Surgical periodontal therapy. *Periodontol 2000*. 2001;25:89-99. doi:10.1034/j.1600-0757.2001.22250107.x

18. Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical treatment of human periodontal disease. *J Clin Periodontol*. Jul 1982;9(4):290-6. doi:10.1111/j.1600-051x.1982.tb02095.x

19. Ali M, Yang F, Plachokova AS, Jansen JA, Walboomers XF. Application of specialized pro-resolving mediators in periodontitis and peri-implantitis: a review. *Eur J Oral Sci*. Feb 2021;129(1):e12759. doi:10.1111/eos.12759

20. Van Dyke TE, Lester MA, Shapira L. The role of the host response in periodontal disease progression: implications for future treatment strategies. *J Periodontol*. Aug 1993;64(8 Suppl):792-806. doi:10.1902/jop.1993.64.8s.792

21. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE2 secretion as a determinant of periodontal disease expression. *J Periodontol*. May 1993;64(5 Suppl):432-44.

doi:10.1902/jop.1993.64.5s.432

22. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol*. Dec 2018;16(12):745-759. doi:10.1038/s41579-018-0089-x

23. Curtis MA, Diaz PI, Van Dyke TE. The role of the microbiota in periodontal disease. *Periodontol 2000*. Jun 2020;83(1):14-25. doi:10.1111/prd.12296

24. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast Cell: A Multi-Functional Master Cell. *Front Immunol*. 2015;6:620. doi:10.3389/fimmu.2015.00620

25. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol*. Oct 2013;14(10):986-95. doi:10.1038/ni.2705

26. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol*. Mar 2006;6(3):173-82. doi:10.1038/nri1785

27. Nathan C. Points of control in inflammation. *Nature*. Dec 19-26 2002;420(6917):846-52. doi:10.1038/nature01320

28. Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol*. Sep 2018;233(9):6425-6440. doi:10.1002/jcp.26429

29. Dalli J, Serhan CN. Pro-Resolving Mediators in Regulating and Conferring Macrophage Function. *Front Immunol*. 2017;8:1400. doi:10.3389/fimmu.2017.01400

30. Villadangos JA, Schnorrer P, Wilson NS. Control of MHC class II antigen presentation in dendritic cells: a balance between creative and destructive forces. *Immunol Rev*. Oct 2005;207:191-205. doi:10.1111/j.0105-2896.2005.00317.x

31. Bodineau A, Coulomb B, Tedesco AC, Séguier S. Increase of gingival matured dendritic cells number in elderly patients with chronic periodontitis. *Arch Oral Biol*. Jan 2009;54(1):12-6. doi:10.1016/j.archoralbio.2008.06.014

32. Xu XW, Liu X, Shi C, Sun HC. Roles of Immune Cells and Mechanisms of Immune Responses in Periodontitis. *Chin J Dent Res*. Dec 23 2021;24(4):219-230.

doi:10.3290/j.cjdr.b2440547

33. Pan W, Wang Q, Chen Q. The cytokine network involved in the host immune response to periodontitis. *Int J Oral Sci*. Nov 5 2019;11(3):30. doi:10.1038/s41368-019-0064-z

34. Berglundh T, Liljenberg B, Tarkowski A, Lindhe J. The presence of local and circulating autoreactive B cells in patients with advanced periodontitis. *J Clin Periodontol*. Apr 2002;29(4):281-6. doi:10.1034/j.1600-051x.2002.290402.x

35. Taubman MA, Valverde P, Han X, Kawai T. Immune response: the key to bone resorption in periodontal disease. *J Periodontol*. Nov 2005;76(11 Suppl):2033-41. doi:10.1902/jop.2005.76.11-S.2033

36. Gaffen SL, Hajishengallis G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *J Dent Res*. Sep 2008;87(9):817-28. doi:10.1177/154405910808700908

37. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol*. Apr 1 1986;136(7):2348-57.

38. Gemmell E, Seymour GJ. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontol 2000*. 2004;35:21-41. doi:10.1111/j.0906-6713.2004.003557.x

39. Deng J, Sun D, Luo F, et al. Anti-IFN-γ Antibody Promotes Osteoclastogenesis in Human Bone Marrow Monocyte-Derived Macrophages Co-Cultured with Tuberculosis-Activated Th1 Cells. *Cell Physiol Biochem*. 2018;49(4):1512-1522. doi:10.1159/000493455

40. Alroqi FJ, Chatila TA. T Regulatory Cell Biology in Health and Disease. *Curr Allergy Asthma Rep*. Apr 2016;16(4):27. doi:10.1007/s11882-016-0606-9

41. Alvarez C, Rojas C, Rojas L, Cafferata EA, Monasterio G, Vernal R. Regulatory T Lymphocytes in Periodontitis: A Translational View. *Mediators Inflamm*. 2018;2018:7806912. doi:10.1155/2018/7806912

42. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. *Front Immunol*. 2012;3:51. doi:10.3389/fimmu.2012.00051

43. Liberal R, Grant CR, Longhi MS, Mieli-Vergani G, Vergani D. Regulatory T cells: Mechanisms of suppression and impairment in autoimmune liver disease. *IUBMB Life*. Feb 2015;67(2):88-97. doi:10.1002/iub.1349

44. Ferguson B, Bokka NR, Maddipati KR, et al. Distinct Profiles of Specialized Pro-resolving Lipid Mediators and Corresponding Receptor Gene Expression in Periodontal Inflammation. *Front Immunol*. 2020;11:1307. doi:10.3389/fimmu.2020.01307

45. Barden AE, Moghaddami M, Mas E, Phillips M, Cleland LG, Mori TA. Specialised pro-resolving mediators of inflammation in inflammatory arthritis. *Prostaglandins Leukot Essent Fatty Acids*. Apr 2016;107:24-9. doi:10.1016/j.plefa.2016.03.004

46. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol*. Jul 2001;2(7):612-9. doi:10.1038/89759

47. Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. *Blood*. Oct 11 2012;120(15):e60-72. doi:10.1182/blood-2012-04-423525

48. Slots J. Periodontitis: facts, fallacies and the future. *Periodontol 2000*. Oct 2017;75(1):7-23. doi:10.1111/prd.12221

49. Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease? *Periodontol 2000*. Oct 2017;75(1):152-188. doi:10.1111/prd.12201

50. Liu J, Ruan J, Weir MD, et al. Periodontal Bone-Ligament-Cementum Regeneration via Scaffolds and Stem Cells. *Cells*. Jun 4 2019;8(6)doi:10.3390/cells8060537

51. Kapoor A, Malhotra R, Grover V, Grover D. Systemic antibiotic therapy in periodontics. *Dent Res J (Isfahan)*. Sep 2012;9(5):505-15. doi:10.4103/1735-3327.104866

52. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol*. May 2011;31(5):986-1000. doi:10.1161/ATVBAHA.110.207449

53. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. *Aging Dis*. Feb 2018;9(1):143-150. doi:10.14336/AD.2017.0306

54. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. *J Pharm Pharm Sci*. 2013;16(5):821-47. doi:10.18433/j3vw2f

55. Balta MG, Papathanasiou E, Christopoulos PF. Specialized Pro-Resolving Mediators as Potential Regulators of Inflammatory Macrophage Responses in COVID-19. *Front Immunol*. 2021;12:632238. doi:10.3389/fimmu.2021.632238

56. Hasturk H, Schulte F, Martins M, et al. Safety and Preliminary Efficacy of a Novel Host-Modulatory Therapy for Reducing Gingival Inflammation. *Front Immunol*. 2021;12:704163. doi:10.3389/fimmu.2021.704163

57. Ponce J, Ulu A, Hanson C, et al. Role of Specialized Pro-resolving Mediators in Reducing Neuroinflammation in Neurodegenerative Disorders. *Front Aging Neurosci*. 2022;14:780811. doi:10.3389/fnagi.2022.780811

58. Regidor PA, Santos FG, Rizo JM, Egea FM. Pro resolving inflammatory effects of the lipid mediators of omega 3 fatty acids and its implication in SARS COVID-19. *Med Hypotheses*. Dec 2020;145:110340. doi:10.1016/j.mehy.2020.110340

59. Wang RX, Colgan SP. Special pro-resolving mediator (SPM) actions in regulating gastro-intestinal inflammation and gut mucosal immune responses. *Mol Aspects Med*. Dec 2017;58:93-101. doi:10.1016/j.mam.2017.02.002

60. Zahoor I, Giri S. Specialized Pro-Resolving Lipid Mediators: Emerging Therapeutic Candidates for Multiple Sclerosis. *Clin Rev Allergy Immunol*. Apr 2021;60(2):147-163. doi:10.1007/s12016-020-08796-4

61. Abrams DI, Dolor R, Roberts R, et al. The BraveNet prospective observational study on integrative medicine treatment approaches for pain. *BMC Complement Altern Med*. Jun 24 2013;13:146. doi:10.1186/1472-6882-13-146

62. Hasturk H, Kantarci A, Goguet-Surmenian E, et al. Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis in vivo. *J Immunol*. Nov 15 2007;179(10):7021-9. doi:10.4049/jimmunol.179.10.7021

63. Serhan CN, Arita M, Hong S, Gotlinger K. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids*. Nov 2004;39(11):1125-32. doi:10.1007/s11745-004-1339-7

64. Chapkin RS, Kim W, Lupton JR, McMurray DN. Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. *Prostaglandins Leukot Essent Fatty Acids*. Aug-Sep 2009;81(2-3):187-91. doi:10.1016/j.plefa.2009.05.010

65. Kantarci A, Oyaizu K, Van Dyke TE. Neutrophil-mediated tissue injury in periodontal disease pathogenesis: findings from localized aggressive periodontitis. *J Periodontol*. Jan 2003;74(1):66-75. doi:10.1902/jop.2003.74.1.66

66. Van Dyke TE, Hasturk H, Kantarci A, et al. Proresolving nanomedicines activate bone regeneration in periodontitis. *J Dent Res*. Jan 2015;94(1):148-56. doi:10.1177/0022034514557331