

THE IMPORTANCE OF CYTOKINES IN PERIODONTAL DISEASE AND RHEUMATOID ARTHRITIS. REVIEW.

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Abstract

Both rheumatoid arthritis (RA) and periodontitis (PD) are complex chronic inflammatory diseases who share a multitude of susceptibility factors (especially genetic and environmental risk factors). A important difference between these pathologies is that periodontal disease results from the inflammation that develops in response to the subgingival microbiota, while the inflammation in rheumatoid arthritis stems from an exaggerated specific adaptive autoimmune response. This proinflammatory status is perpetuated by the continued bacterial challenge in periodontitis and the autoimmune response triggered in RA, culminating in the progressive destruction of tissues that eventually lead to the signs and symptoms of the disease.

Despite the differences in mechanisms of etiological initiation, the idea of polymorphisms of genes that encode certain cytokines which results in connective tissue damage and bone metabolism alterations in the two pathologies mentioned is a bridge which links these diseases. Additionally, there is evidence that both periodontitis and RA are manifested as persistent levels of proinflammatory cytokines and associated molecules. In addition, therapeutic strategies based on blocking proinflammatory cytokines in RA have been shown to have an impact on the overall periodontal status.

This review analyses the main molecules which have an impact in the development of both diseases and evaluates possible clinical implications and bidirectional relationships which influence the overall status of the patient.

Keywords: *periodontitis, proinflammatory cytokines, rheumatoid arthritis*

Introduction

Periodontal disease is defined as an inflammatory disease of teeth supporting tissues caused by specific microorganisms or groups of specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with the formation of periodontal pockets, gingival recession or both. The clinical feature that distinguishes gingivitis from periodontitis is the presence of a clinically

detectable loss of attachment. This loss is often accompanied by the formation of periodontal pockets and changes in the density and height of the underlying alveolar bone.[1] In some cases, the recession of the marginal gingiva can lead to the loss of attachment, thus concealing the continued progression of the disease if only periodontal scalling is performed as treatment, without checking the clinical attachment.[2,3] Clinical signs of inflammation - such as color changes, contours and consistency, and bleeding

on probing - may not always be positive indicators of the ongoing, progressive attachment loss.[4] More modern methods of quantifying the disease progression are inflammation and bone loss biomarkers and also PCR for identifying key periodontopathogens.[5,6]

Recent findings place a more important role in the etiology and development of periodontal disease on viruses.[7] Besides bacteria, herpesviruses that originate in the periodontal tissues can disseminate through the blood stream to other organs and may represent an important link in the periodontitis – systemic diseases binome.[8] In this context treatment of periodontal disease that concentrates mainly on microbial biofilm eradication will require a revision in order to grant sustainable clinical improvement and possibly reduce the risk of systemic diseases.[9]

Treatment of this disease is often misconducted leading to the subsequent loss of teeth. If dental implantation is performed without thoroughly disinfecting the implantation situs chances of success are greatly diminished.[10]

Rheumatoid arthritis is a common inflammatory systemic disease characterized by the presence of a destructive polyarthritis with a tendency to affect small joints of the hands, legs and joints (although they may affect any synovial joint).[11] Diagnosis is based on cumulative clinical observations and paraclinical examinations.[12] Rheumatoid arthritis involves a complex interaction between genotype, environmental trigger and chance. It involves a complex interplay among genotype, environmental triggers, and chance. Twin studies implicate genetic factors in rheumatoid arthritis, also genomewide analyses make it clear that regulatory factors of the immune system underlie the disease. The long-established association with the human leukocyte antigen (HLA)–DRB1 locus has been confirmed in patients who are positive for rheumatoid factor or ACPA.[13]

Researchers suggest that some predisposing T-cell selection mechanisms, antigen presentation, or modifications in peptide affinity plays a part in promoting a hyperactive

adaptive immune response which is driven towards self-tissues.[14-16] Other possible explanations for the link between rheumatoid arthritis and the shared epitope include molecular mimicry of the shared epitope by microbial proteins, increased T-cell senescence induced by shared epitope-containing HLA molecules, and a potential proinflammatory signaling function that is unrelated to the role of the shared epitope in antigen recognition.[17,18]

The importance of the immune system in the two pathologies

Unlike the specific immune responses that take days to mobilize after exposure to antigens, innate immunity consists in protection against infections that are ready for immediate action when a host is attacked by a pathogen (viruses, bacteria, fungi or parasites).[19]

The innate immune system includes anatomical barriers to infection - both physical and chemical responses, as well as cellular responses. Immune cellular responses inherited from an infective agent's invasion that exceeds the initial epithelial barriers are fast, usually starting a few minutes after the invasion. These responses are triggered by cellular or intracellular surface receptors that recognize the conserved molecular components of pathogens.[20] Some types of white blood cells (macrophages and neutrophils) are activated to swallow rapidly and destroy extracellular microbes through the phagocytosis process. Other receptors induce protein production and other substances that have a variety of beneficial effects, including direct antimicrobial activity and recruitment of fluid cells, cells and molecules at infection sites. This influx causes swelling and other physiological changes collectively called inflammation. Such innate and inflammatory local responses are usually beneficial for eliminating pathogens and dead or damaged cells and promoting healing.[21] For example, increased levels of antimicrobial and phagocytic cells help eliminate pathogens and dendritic cells acquire pathogens for presentation to lymphocytes by activating adaptive immune responses. Natural killer cells recruited on the site can recognize and kill cells

infected with virus, altered or stressed. [22] However, in some situations, these innate and inflammatory responses can be detrimental, resulting in local or systemic consequences that can cause tissue damage and sometimes death. In order to prevent these potentially harmful reactions, regulatory mechanisms have evolved, mechanisms which usually limit such adverse effects. [23]

The molecules that communicate between the cells of the immune system are called cytokines. Generally, cytokines are soluble molecules, although some also exist in membrane-bound forms. In an early attempt to classify cytokines, immunologists began to number them in order of their discovery and call them interleukins. This name reflects the fact that interleukins communicate between white blood cells (leukocytes).[24] Examples include macrophage secreted interleukin 1 (IL-1) and interleukin 2 (IL-2) secreted by activated T cells. However, numerous cytokines previously named, before this nomenclature rationalization attempt do not fit into the desired pattern, and thus cytokines such as tumor necrosis factor or interferons, which are also "interleukins", may be encountered although the name does not suggest it.

Cytokines regulate the intensity and duration of immune response by stimulating or inhibiting the activation, proliferation and / or differentiation of different cells by regulating the secretion of other cytokines or antibodies or, in some cases, by effectively inducing the death of the programmed cells in the target cell. In addition, cytokines can modulate the expression of different cell surface receptors for chemokines, other cytokines or for themselves. Thus, cytokines secreted by a small number of antigen-activated lymphocytes can influence the activity of many different types of cells involved in the immune response.[25] Cytokines exhibit pleiotropy, redundancy, synergism, antagonism, and cascade induction attributes that enable them to regulate cellular activity in a coordinated manner. A cytokine that induces differentiated biological effects depending on the nature of the target cells is considered to have pleiotropic action, while two or more cytokines mediating similar functions are

considered to be redundant.[26] Synergy of cytokines occurs when the combined effect of two cytokines on cellular activity is greater than the effects of individual cytokines. In some cases, the effects of a cytokine inhibit or antagonize the effects of another. Cascade induction occurs when the action of a cytokine on a target cell induces that cell to produce one or more additional cytokines.[27]

Cytokines in periodontal disease

Although parodontitis pathogenesis has traditionally focused on the role of bacterial infection, over the past two decades the interest in host response factors leading to periodontal disease (PD) has increased. It is now understood that immune and inflammatory responses are critical to the pathogenesis of periodontitis and are modeled by a number of host-related factors, both intrinsic (e.g., genetics) and induced (e.g., pollutants).[28,29]

Certain microbial factors (lipopolysaccharides, peptidoglycans, lipoteichoic acids, proteases but also toxins) provoke the inflammatory response and can be localised in the biofilm of tooth surfaces.[30] The host response to the microbial challenge encompasses the action and stimulation of various proinflammatory cell types as well as of resident cells of the tissue.[31]

The initial response to bacterial infection is a local inflammatory reaction that activates the innate immune system. Amplification of this initially localized response results in the release of a series of cytokines and other mediators and the spread of inflammation through the gingival tissues.[32] Failure to encapsulate this "inflammatory assault" in gingival tissue leads to the expansion of the response adjacent to the alveolar bone. The inflammatory process then leads to the destruction of connective tissue and alveolar bone, which is the cardinal sign of PD.[33]

Whether bone loss will occur in response to an inflammatory reaction, it is now known that it depends on two critical factors. First, the concentration of inflammatory mediators present in gingival tissue should be sufficient to activate the pathways that lead to

bone resorption. Secondly, inflammatory mediators must penetrate the gingival tissue to reach a critical distance from the alveolar bone.[34] Achieving critical concentrations of inflammatory mediators leading to bone resorption depends on the expression of proinflammatory cytokines such as, but not limited to interleukin (IL) -1, -6, -11 and -17, tumor necrosis factor alpha (TNF- α), leukemic inhibitory factor and M oncostatin.[35,36]

Kinins, such as bradykinin, thrombin, and various chemokines also have a stimulating effect on bone resorption. This is the opposite of the expression of anti-inflammatory cytokines and other mediators such as IL-4, -10, -12, -13 and -18, as well as interferon-beta (IFN- β) and gamma (IFN- γ), that serve to inhibit bone resorption.[37] In children with insulin dependent diabetes mellitus however, some authors found that IL-18 has increased values compared to healthy controls, thus acting as a proinflammatory marker.[38]

When CD4 T helper cells (Th0) interact with antigen-presenting cells, they differentiate into different possible subsets such as Th1, Th2, Th17 and regulatory T cells (Tregs), according to the cytokines which they generate. Th1 initiate the immune response mediated by cells and produce interferon- γ (IFN- γ), transforming growth factor- β (TGF- β), interleukin-2 (IL-2) and TNF α in the presence of IL-12. Th2 advocate the humoral immune response and generate IL-4, IL-5, IL-6, IL-10, IL-13 and TGF- β in the presence of IL-4. The remaining CD4 T cells, Th17 and Tregs usher a critical role in autoimmunity and in the perpetuation of immune homeostasis. The TH17 subset secrete IL-17, IL-23, IL-22, IL-6 and TNF α in the presence of TGF- β , IL-1 β and IL-6, whereas Tregs emerge in the presence of TGF- β and produce IL-10 and TGF- β which are immunosuppressive.[39]

Particularly, IL-17 enhances the production of various proinflammatory molecules including TNF α , prostaglandin E2 (PGE2), IL-6 and IL-1 β , facilitating bone resorption via osteoclast activation. Altered regulation of the immune system by Treg cells, thought to mediate the resolution of inflammation, is partially responsible for the

pathogenesis of several autoimmune diseases, such as rheumatoid arthritis (RA), lupus, colitis and multiple sclerosis.[40,41]

Inflammation and bone loss are distinctive signs of periodontal disease (PD). The question is how the former determines the latter. Accumulated evidence demonstrates that PD involves bacterial and antigen-derived factors that stimulate a local inflammatory response and activation of the non-specific immune system. Proinflammatory molecules and cytokine networks play an essential role in this process. Interleukin-1 and tumor necrosis factor-alpha appear to be the primary molecules that in turn influence the cells in the lesion site. Antigen-stimulated lymphocytes (B and T cells) also appear to be important.

Eventually, a cascade of events leads to osteoclastogenesis and subsequent bone loss through the RANKL-receptor-ligand receptor (RANKL) - osteoprotegerin (OPG) -RANK.[42] This axis and its regulation are not unique to PD, but rather critical for pathological lesions that involve chronic inflammation.

Multiple evidence in PD models clearly indicate that increases in RANKL mRNA expression and protein production increase the RANKL/OPG ratio and stimulate the differentiation of macrophage precursor cells into osteoclasts. They also stimulate the maturation and survival of osteoclasts, leading to bone loss.[43]

The fact that proinflammatory cytokines are integrated in the propagation of the inflammatory response to bone proximal regions has been demonstrated in a study of experimental periodontitis on *Macaca fascicularis* primate model. In this animal model, silk ligatures imbedded with *Porphyromonas gingivalis* (Pg) were applied to the mandibular posterior teeth to induce experimental periodontitis. Primates received local injections for 6 weeks with TNF- α and IL-1 antagonists (soluble TNF- α and IL-1 receptors). Analysis of gingival connective tissue sections in the immediate vicinity of the bone demonstrated significant recruitment of inflammatory cells and formation of osteoclasts surrounding the bone in control primates.[44]

During an inflammatory response, cytokines, chemokines and other mediators stimulate periosteal osteoblasts by altering the expression levels of a protein called factor-kappa B (RANKL) receptor activator on the osteoblast surface. RANKL is expressed by osteoblasts in the protein membrane bound or split into a soluble form. [43] In addition to osteoblasts, RANKL is expressed by a number of other cell types, including fibroblasts and T and B lymphocytes. RANKL is expressed at low levels in fibroblasts; however, its expression is induced as a response to the cytotoxic substance of *Aggregatibacter actinomycetemcomitans*. [45]

Cytokines in rheumatoid arthritis

One of the hallmarks of RA is the persistent synovitis resulting from the immune cell influx into the joints. This pathology is more and more recognized to amount to a more expansive syndrome that contains high end cardiovascular morbidity, psychological impairment, risk of cancer and osteoporosis. [46] The past decade has seen the advent of novel therapeutics in the form of both biologic agents and small-molecules. At least as important as these unparalleled advances in drug development has been the introduction of strategic management approaches that have made remission or low disease activity the target of treatment. [47,48]

Infiltration of the synovial membrane and fluid with leukocytes is defined primarily by cells of the innate immune system. Thus, macrophages, mast cells, natural killer (NK) cells and neutrophils have been described as crucial components of the synovial infiltrate and their effector functions clearly link to disease manifestations. More recently, innate lymphoid cell lineages have been characterized that further extend the contribution of innate effector components to tissue destruction. Synovitis is characterized by wide cellular expression of damage-associated molecular patterns and pathogen-associated molecular patterns that facilitate dysregulated activation of these various cell lineages, in the presence of chronic damage but without recourse to specific antigen. Critically, the kinetics of immune function in

RA tissues are unlikely to be 'synchronized' by an initiating event and, as such, the normal sequential regulatory homeostasis that is integral to classical innate immune responses in relation to antigen-triggered responses is unlikely to operate in the rheumatoid joint. From the pathological perspective, the net effect of this cellular profile is the generation of tissue-destructive enzymes, reactive oxygen and nitrogen intermediates, prostaglandins and leukotrienes, and a broad range of effector cytokines, outside their normal homeostatic 'on-off' regulatory cycle. [49]

In this context, effector T cells, together with B cells and other effector cells, form a complex network that promotes the production of pro-inflammatory cytokines, triggering the activation of fibroblast-like synoviocytes and contributing to bone and cartilage lesions. Innate immune cells such as neutrophils and mast cells contribute to the development of synovitis, as well as macrophages, which function by the release of proinflammatory cytokines (such as TNF, IL-1 and IL-6) and inflammatory mediators such as free oxygen radicals, nitrogen intermediates and prostanoids. [50]

For many years, the dominant dogma was that the macrophages polarize in the pro-inflammatory phenotype "M1" in the AR, resulting in the production of pro-inflammatory mediators and a reduction in control mechanisms and anti-inflammatory cytokines such as growth factor type TGF β , IL-4, IL-13 and IL-10. However, phenotypes from synovial macrophages in AR patients are diverse and do not follow a strict M1 or M2 phenotype. It is debatable which of the cytokines have only anti- or pro-inflammatory functions, but it has been observed that they function in a complex network dependent on the specific pathological and tissue context. [51]

The IL-1 family consists mainly of IL-1 α , IL-1 β , IL-18 and IL-33. IL-1 α is either expressed on the cell surface or is contained within the cell, while IL-1 β produces its biological activity by acting on other cells. The action of IL-1 α And IL-1 β may be blocked by an endogenous inhibitor, antagonist of IL-1

receptor (IL-1Ra). Patients with RA show an imbalance between IL-1Ra and IL-1 levels.[52]

In many cases a high concentration of IL-1 β in plasma and synovial fluid (SF) has been found, which explains various parameters of disease activity, including duration of stiffness.

The cytokine IL-2 is crucial to the function, expansion and survival of regulatory T cells (Treg) and equilibrium in this way is disrupted in Th1 helper cell-mediated autoimmune diseases such as type 1 diabetes mellitus, RA and erythematous systemic lupus. In a study from 2018, patients with RA had elevated levels of anti-IL-2, the authors assuming that they would affect the bioavailability of IL-2 required for Treg homeostasis.[53]

Interleukin-4 is a major cytokine that promotes the generation of Th2 cells by differentiating naive T cells. Through the positive response loop, IL-4 is further generated by activated Th2 cells. IL-4Ra is the IL-4 receptor that exists in various forms in the body. Initially, it was reported to be either absent or present in very small amounts in the synovial fluid of patients with AR, but was detected in some RA patients. It is an anti-inflammatory cytokine that prevents the formation of proinflammatory cytokines such as TNF- α , IL-1 β and IL-6 in synovial tissue and peripheral blood mononuclear cells (PBMCs). In vitro studies have shown that IL-4 reduces bone resorption by having a direct influence on osteoclasts and may also inhibit the production of MMP.[54]

TNF and IL-6 are considered key points of the synovial system in the RA, which stimulates the formation and subsequent degradation of bone and cartilage and also strongly induce the release of other pro-inflammatory mediators such as IL-1 and stimulatory factor granulocyte-macrophage colonies - GM-CSF.

Targeting TNF and IL-6 with neutralizing antibodies or, in the case of IL-6, with Janus kinase inhibitors (JAK), is a strategy now widely used in the treatment of RA, which can effectively suppress synovial inflammation. Furthermore, GM-CSF inhibition has been

effective in the treatment of RA in early-stage clinical trials and is currently awaiting approval for clinical use.[55]

The role in pathology of IL-6 has been documented in patients with rheumatoid arthritis (RA); to them, there was a tendency of increase in the level of IL-6 in GCF. Thus, one study indicated higher IL-6 serum values in RA patients compared to patients without systemic disease, but comparable to age-type, gender-, smoking-status variables, IL-6 serum levels showing a positive correlation with activity RA. These observations suggest that systemically and locally produced IL-6 may play a role in regulating periodontal inflammation in RA patients.[56]

Interleukin-17 is of particular interest for the pathogenesis of periodontitis due to its involvement in both inflammation and antimicrobial immunity, some research confirming that IL-17 mediates protection against extracellular pathogens and, together with IL-22 (a cytokine produced also by Th17 and by other IL-17 expressing cells) production of antimicrobial peptides (AMP), which is believed to be protective in periodontitis.[57,58]

In principle, IL-17 is a two-edged paradigm sword for a disease such as periodontitis that is initiated by bacteria, although tissue damage is caused by the host's response (de Aquino et al., 2017). Therefore, the biological properties of IL-17 make it difficult to estimate its role in inflammatory diseases with a polymicrobial etiology. It is possible that IL-17 exerts both protective and destructive effects as suggested in distinct mouse models, although signaling of the IL-17 receptor may turn an acute protective inflammatory response into chronic immunopathological events.[59]

Although it is not known exactly how periodontal bacteria can regulate IL-17 production, there is evidence that *P. gingivalis* promotes an IL-17 medium apparently to exploit the resulting inflammatory response in order to obtain nutrients in the form of decomposition products of tissues and hem molecules.[60]

In this regard, *P. gingivalis* stimulation of PBMC in healthy volunteers led to an increase in IL-17 production in CD3 + cells and an increase in IL-23 production in

macrophages. Furthermore, the isolated lipopolysaccharide from *P. gingivalis* has been shown to induce IL-17 and IL-23 production from human periodontal ligation cells, while external membrane proteins can stimulate mRNA expression for IL-17 in PBMC isolated from patients with gingivitis or periodontal disease.[61]

In part, the suppression of Th1 cell-mediated immunity by *P. gingivalis* could be attributed to its ability to inhibit the production of chemokine in the gingival epithelial cells responsible for Th1 recruitment. Generally, *P. gingivalis* has an arsenal of virulence factors by which it can manipulate specific and non-specific immune cells to initiate an IL-17 orchestrated rich inflammatory response.[62]

Importantly, the presence of *P. gingivalis* in subgingival biofilm was associated with increased IL-17 levels in crevular fluid in periodontitis.[63]

Continuing advancement in scientific methodology, including high throughput analysis techniques, is enabling studies on genomic variations and gene expression patterns in periodontal disease and rheumatoid arthritis. Whole-genome microarrays and RNA sequencing will be valuable tools for identifying genetic and biological markers of increased susceptibility to both pathologies shedding light into the possible implications and interdependencies. In recent years, both transcriptome studies and a genome-wide association study have been performed on periodontitis cohorts.[64,65] The massive amounts of data generated by such studies require painstaking analyses to yield biologically significant results, but the capacity for

identification of novel mediators involved in the pathogenesis of both diseases is promising.[66]

However, upcoming breakthroughs in the understanding and treatment of these pathologies need not be derived only from periodontitis or arthritis focused research. Much is to be gained from research progress in other chronic inflammatory conditions. Studies are ongoing to evaluate the role of other proinflammatory cytokines in the pathophysiology of similar conditions, such as lupus, Crohn's disease, Alzheimer's and irritable bowel disease, and to develop antibodies which specifically target these cytokines for novel future treatment strategies.

Conclusion

As discussed throughout this review, research into the molecular pathogenesis of periodontitis and rheumatoid arthritis is continuously producing novel and significant results. Despite extensive research, however, the detailed mechanisms of pathogenesis are still not elucidated. Nevertheless, the field is moving forward, utilising technological advances and synergy effects from findings in closely related diseases. Periodontitis is currently being connected to the pathogenesis of various systemic diseases and conditions, further emphasising the importance of a deeper understanding of this common condition. Progress in the understanding of periodontal disease may enable adjunctive treatments focused on modulating the host response. Successful novel treatment strategies have the potential to improve both the oral and the systemic health of patients afflicted with periodontitis.

References:

1. Caton JG, Armitage G, Berglundh T, Chapple IL, Jepsen S, Kornman KS, Mealey BL, Papapanou PN, Sanz M, Tonetti MS. A new classification scheme for periodontal and peri-implant diseases and conditions—Introduction and key changes from the 1999 classification. *Journal of periodontology*. 2018 Jun;89:S1-8.
2. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nature Reviews Disease Primers*. 2017 Jun 22;3:17038.

3. Șufaru IG, Solomon SM, Păsărin L, Martu-Stefanache A, Oanță C, Ciocan-Pendefunda A, Mârțu Silvia. Study regarding the quantification of RANKL levels in patients with chronic periodontitis and osteoporosis. *Rom. J of Oral Rehab*, 2016,8(4):47-51.
4. Solomon S, Gianina I, Liliana P, Georgeta SI, Ioana M, Ionuț L, Alexandra MM, Silvia M. Risk predictors in periodontal disease. *Romanian Journal of Oral Rehabilitation*. 2017 Jul 1;9(3):89-96.
5. Solomon SM, Matei MN, Badescu AC, Jelihovschi I., Martu-Stefanache A., Teusan A., Martu S., Iancu LS..Evaluation of DNA Extraction Methods from Saliva as a Source of PCR – Amplifiable Genomic DNA. *Rev. Chim. (Bucharest)*, 2015, 66(12):2101-2103.
6. Popa C, Stelea C, Filioreanu AM, Grigoras SI, Sufaru IG, Maftei GA, Scutariu MM, Martu S, Popescu E. The assessment of the association between herpesviruses and subgingival bacterial plaque by real-time PCR analysis. *Rev. Chim. (Bucharest)*. 2017; 68(11): 2672-2675
7. Slots J, Slots H. Periodontal herpesvirus morbidity and treatment. *Periodontology* 2000. 2019 Feb;79(1):210-20.
8. Solomon SM, Filioreanu AM, Stelea CG, Grigoras S, Sufaru IG, Maftei GA, Martu S, Scutariu MM, Popa C. The Assessment of the Association Between Herpesviruses and Subgingival Bacterial Plaque by Real-time PCR Analysis. *Rev. Chim.(Bucharest)*, 2018; 69(2):507-510
9. Nicolae V, Chiscop I, Cioranu Ibric VS, Martu MA, Luchian AI, Martu S, Solomon SM, The Use of Photoactivated Blue-O Toluidine for Periimplantitis Treatment in Patients with Periodontal Disease. *Rev Chim (Bucharest)* 2015; 66(12):2121-2123.
10. Forna NC, Perez J, Pilipili C, Songo F, Forna DA. THE USE OF Er, Cr: YAG IN THE TREATMENT OF PERI-IMPLAANTITIS. *Romanian Journal of Oral Rehabilitation*. 2018 Jul 1;10(3):15-21.
11. Angelotti F, Parma A, Cafaro G, Capecchi R, Alunno A, Puxeddu I. One year in review 2017: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol*. 2017 May 1;35(3):368-78.
12. Macovei LA, Rezuș E. Cervical spine lesions in rheumatoid arthritis patients. *Rev Med Chir Soc Med Nat Iasi*. 2016 Jan 1;120(1):70-6.
13. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *New England Journal of Medicine*. 2011 Dec 8;365(23):2205-19.
14. Catrina AI, Svensson CI, Malmström V, Schett G, Klareskog L. Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. *Nature Reviews Rheumatology*. 2017 Feb;13(2):79.
15. Boissier MC, Semerano L, Challal S, Saidenberg-Kermanac'h N, Falgarone G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. *Journal of autoimmunity*. 2012 Sep 1;39(3):222-8.
16. Hueber AJ, Asquith DL, Miller AM, Reilly J, Kerr S, Leipe J, Melendez AJ, McInnes IB. Cutting edge: mast cells express IL-17A in rheumatoid arthritis synovium. *The Journal of Immunology*. 2010 Apr 1;184(7):3336-40.
17. Scher JU, Abramson SB. The microbiome and rheumatoid arthritis. *Nature Reviews Rheumatology*. 2011 Oct;7(10):569.
18. Martu MA, Solomon SM, Sufaru IG, Jelihovschi I, Martu S, Rezus E, Surdu AE, Onea RM, Grecu GP, Foia L. Study on the prevalence of periodontopathogenic bacteria in serum and subgingival bacterial plaque in patients with rheumatoid arthritis. *Rev. Chim.(Bucharest)*. 2017 Aug 1;68(8):1946-9.
19. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012 Jun 8;336(6086):1268-73.
20. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *science*. 2010 Jan 15;327(5963):291-5.
21. Turvey SE, Broide DH. Innate immunity. *Journal of Allergy and Clinical Immunology*. 2010 Feb 1;125(2):S24-32.

22. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nature immunology*. 2015 Apr;16(4):343.
23. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nature medicine*. 2012 Mar;18(3):363.
24. Paul WE, Seder RA. Lymphocyte responses and cytokines. *cell*. 1994 Jan 28;76(2):241-51.
25. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nature reviews immunology*. 2011 Nov;11(11):723.
26. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Current opinion in immunology*. 2010 Aug 1;22(4):507-13.
27. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nature immunology*. 2010 Sep;11(9):785.
28. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontology 2000*. 2014 Feb;64(1):57-80.
29. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology*. 2015 Jan;15(1):30.
30. Nishihara T, Koseki T. Microbial etiology of periodontitis. *Periodontology 2000*. 2004 Oct;36(1):14-26.
31. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontology 2000*. 1997 Jun;14(1):12-32.
32. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. *Periodontology 2000*. 2015 Oct;69(1):7-17.
33. Hienz SA, Paliwal S, Ivanovski S. Mechanisms of bone resorption in periodontitis. *Journal of immunology research*. 2015;2015.
34. Siqueira JF, Rôças IN. The oral microbiota in health and disease: an overview of molecular findings. *InOral Biology 2017* (pp. 127-138). Humana Press, New York, NY.
35. Kurgan S, Kantarci A. Molecular basis for immunohistochemical and inflammatory changes during progression of gingivitis to periodontitis. *Periodontology 2000*. 2018 Feb;76(1):51-67.
36. Solomon S, Pasarin L, Ursarescu I, Martu I, Bogdan M, Nicolaiciuc O, Ioanid N, Martu S. The effect of non-surgical therapy on C Reactive Protein and IL-6 serum levels in patients with periodontal disease and atherosclerosis. *Int. J. of Cl and Experimental Medicine*, 2016;9(2):4411-4417.
37. Preshaw PM. Host modulation therapy with anti-inflammatory agents. *Periodontology 2000*. 2018 Feb;76(1):131-49.
38. Toma V, Cioloca DP, Forna DA, Hurjui L, Botnariu GI, Nechifor IE, Bogdan M, Costuleanu M, Simion L, Holban C. IL 18 as an important gingival inflammatory biochemical marker in children and adolescents with insulin-dependent diabetes mellitus. *Rev. Chim.(Bucharest)*. 2016 Dec 1;67(12):2545-51.
39. Nakayamada S, Takahashi H, Kanno Y, O'Shea JJ. Helper T cell diversity and plasticity. *Current opinion in immunology*. 2012 Jun 1;24(3):297-302.
40. Afzali B, Lombardi G, Lechler RI, Lord GM. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. *Clinical & Experimental Immunology*. 2007 Apr;148(1):32-46.
41. Nicolaiciuc O, Mihai C, Sufaru IG, Mârțu I, Solomon SM, Tatarciuc D, Rezus C, Mârțu S. Study on the TNF- α , IL-1 β and IL-6 Levels in Patients with Chronic Periodontitis and Cardiovascular Disease. *Rev. Chim. (Bucharest)*, 2017, 68(3): 619-623
42. Teodorescu AC, Martu I, Teslaru S, Kappenberg-Nitescu DC, Goriuc A, Luchian I, Martu MA, Solomon SM, Mârțu S. Assessment of Salivary Levels of RANKL and OPG in Aggressive versus Chronic Periodontitis. *J of Immunology Res, Hindawi*, 2019, Article ID 6195258, 6 pg. <https://doi.org/10.1155/2019/6195258>
43. Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. *Journal of clinical periodontology*. 2012 Mar;39(3):239-48.

44. Ebersole JL, Brunsvold M, Steffensen B, Wood R, Holt SC. Effects of immunization with *Porphyromonas gingivalis* and *Prevotella intermedia* on progression of ligature-induced periodontitis in the nonhuman primate *Macaca fascicularis*. *Infection and immunity*. 1991 Oct 1;59(10):3351-9.
45. Herbert BA, Novince CM, Kirkwood KL. *Aggregatibacter actinomycetemcomitans*, a potent immunoregulator of the periodontal host defense system and alveolar bone homeostasis. *Molecular oral microbiology*. 2016 Jun;31(3):207-27.
46. Leech MT, Bartold PM. The association between rheumatoid arthritis and periodontitis. *Best Practice & Research Clinical Rheumatology*. 2015 Apr 1;29(2):189-201.
47. Calabresi E, Petrelli F, Bonifacio AF, Puxeddu I, Alunno A. One year in review 2018: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol*. 2018 Mar 1;36(2):175-84.
48. Martu MA, Rezus E, Popa C, Solomon SM, Luchian I, Pendefunda AC, Sioustis I, Anton D, Martu S, Foia L. Correlations between systemic therapy with conventional (synthetic) and biological DMARDs, rheumatoid arthritis and periodontal indices of chronic periodontitis. *Rom J of Oral Rehab*, 2018, 10(4):161-169
49. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, Berdelou A, Varga A, Bahleda R, Hollebecque A, Massard C. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *European journal of cancer*. 2016 Feb 1;54:139-48.
50. McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis—shaping the immunological landscape. *Nature Reviews Rheumatology*. 2016 Jan;12(1):63.
51. Chen YM, Chang CY, Chen HH, Hsieh CW, Tang KT, Yang MC, Lan JL, Chen DY. Association between autophagy and inflammation in patients with rheumatoid arthritis receiving biologic therapy. *Arthritis research & therapy*. 2018 Dec;20(1):268.
52. Dayer JM. From supernatants to cytokines: a personal view on the early history of IL-1, IL-1Ra, TNF and its inhibitor in rheumatology. *Arthritis research & therapy*. 2018 Dec;20(1):101.
53. Bo M, Niegowska M, Erre GL, Piras M, Longu MG, Manchia P, Manca M, Passiu G, Sechi LA. Rheumatoid arthritis patient antibodies highly recognize IL-2 in the immune response pathway involving IRF5 and EBV antigens. *Scientific reports*. 2018 Jan 29;8(1):1789.
54. Mateen S, Zafar A, Moin S, Khan AQ, Zubair S. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clinica Chimica Acta*. 2016 Apr 1;455:161-71.
55. Baker KF, Isaacs JD. Novel therapies for immune-mediated inflammatory diseases: What can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis?. *Annals of the rheumatic diseases*. 2018 Feb 1;77(2):175-87.
56. Cosgarea R, Tristiu R, Dumitru RB, Arweiler NB, Rednic S, Sirbu CI, Lascu L, Sculean A, Eick S. Effects of non-surgical periodontal therapy on periodontal laboratory and clinical data as well as on disease activity in patients with rheumatoid arthritis. *Clinical oral investigations*. 2019 Jan 29;23(1):141-51.
57. Schulz S, Pütz N, Jurianz E, Schaller HG, Reichert S. Are There Any Common Genetic Risk Markers for Rheumatoid Arthritis and Periodontal Diseases? A Case-Control Study. *Mediators of inflammation*. 2019;2019.
58. Cheng Z, Meade J, Mankia K, Emery P, Devine DA. Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis. *Best Practice & Research Clinical Rheumatology*. 2017 Feb 1;31(1):19-30.
59. Abusleme L, Moutsopoulos NM. IL-17: overview and role in oral immunity and microbiome. *Oral diseases*. 2017 Oct;23(7):854-65.
60. Bartold PM, Marshall RI, Haynes DR. Periodontitis and rheumatoid arthritis: a review. *Journal of periodontology*. 2005 Nov;76:2066-74.
61. Hajishengallis G, Krauss JL, Jotwani R, Lambris JD. Differential capacity for complement receptor-mediated immune evasion by *Porphyromonas gingivalis* depending on the type of innate leukocyte. *Molecular oral microbiology*. 2017 Apr;32(2):154-65.

62. Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. *Nature Reviews Rheumatology*. 2017 Oct;13(10):606.
63. Mikuls TR, Payne JB, Yu F, Thiele GM, Reynolds RJ, Cannon GW, Markt J, McGowan D, Kerr GS, Redman RS, Reimold A. Periodontitis and *Porphyromonas gingivalis* in patients with rheumatoid arthritis. *Arthritis & rheumatology*. 2014 May;66(5):1090-100.
64. Santiago-Rodriguez TM, Naidu M, Abeles SR, Boehm TK, Ly M, Pride DT. Transcriptome analysis of bacteriophage communities in periodontal health and disease. *BMC genomics*. 2015 Dec;16(1):549.
65. Keschull M, Hülsmann C, Hoffmann P, Papapanou PN. Genome-wide analysis of periodontal and peri-implant cells and tissues. In *Oral Biology 2017* (pp. 307-326). Humana Press, New York, NY.
66. Martu, A; Rezus, E; Sufaru, I; Banu C, Martu Silvia, Foia L Study on the clinical changes in general and oral status in patients with rheumatoid arthritis. *Rom. J. of Oral Rehab.*, 2018,10(3):188-198.