Effect of Tumor Necrosis Factor Alpha (Tnf-A) And Interleukin-10 (II-10) Levels of Aggressive Periodontitis In Rats (*Rattus Norvegicus*) Induced by *Agrregitibacter Actinomycetemcomitans*

Dwi Leni Yuliana[1], Yoes Prijatna Dachlan[2] and Heny Arwati[3]

[1]*Post graduate school Universitas Airlangga, Surabaya, Indonesia*
[2]*Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia*
[3]*Departement parasitology faculty of medicine Universitas Airlangga, Surabaya, Indonesia*

Keywords: Aggregatibacter actinomycetemcomitans, Tumor Necrosis Factor Alpha (TNF-α), Interleukin 10 (IL-10), Agressive Periodontitis.

Abstract: Aggregatibacter actinomycetemcomitans (A.actinomycetemcomitans) is a gram negative and a major bacterial agent associated with aggressive periodontitis in young adults. These bacteria are an important factor in pathogenesis of aggressive periodontitis. A. actinomycetemcomitans possesses fimbriae with an adhesin protein that was is the first bacterial molecules to make physical contact with host. A complex network of pro- and anti-inflammatory cytokines act in inflamed periodontal tissues. Among other cytokines, interleukin-10 (IL-10) is an important multifunctional cytokine. An increase or decrease in IL-10 levels caused by bacterial infection is critical for the individual control of balance between inflammatory, humoral and microbial challenges. Tumor necrosis factor-α (TNF-α) plays an important role in periodontal inflammation as it has substantial potential to increase bone resorption and is involved in connective tissue degradation by stimulating prostaglandin-E2 and colagenase. The purpose of this research was to analyze TNF-α and IL-10 levels of aggressive periodontitis in rats (Rattus norvegicus) induced by A. actinomycetemcomitans. This research was a true experimental study with Post Test Only Control Group Design. Rats were divided into 4 groups for 0,25; 0.5 and 0.75 CFU/mL, and negative control. Each group contained 5 rats. Aggressive periodontitis in rats was induced by injecting A.actinomycetemcomitans, at 48 hrs and 96 hrs post injection the inflammatory signs were observed, thee days later plasma were then collected to measure TNF-α dan IL-10 level by ELISA. Analysis of Variance (ANOVA) showed significantly increase levels of TNF-α in the infected group compared with that of the control group. Aggressive periodontitis in rats showed by redness, abscess and tooth mobility. This condition indicated that A.actinomycetemcomitans has the ability to adhere and invade the periodontal tissue further producing a colony that caused periodontitis. High plasma level of TNF was seen in rats infected with 0.75 CFU/mL OF ac, while IL-10 was low as seen in rats infected with 0.5 CFU/mL OF Ac.

1 INTRODUCTION

Periodontal disease and dental caries are the most prevalent infections affecting the human dentition (Brown et al., 1996). Periodontal disease is a chronic bacterial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction (Yamamoto et al., 2011). Periodontitis, which is bacterially induced, can be defined as a chronic inflammatory disease initiated by dental plaque biofilm and perpetuated by a deregulated immune response (Suvan et al., 2011)

usually accompanied by gingivitis resulting in irreversible destruction of the connective tissues that support the tooth, including the alveolar bone (Yamamoto et al., 2011).

The gingiva, periodontium, alveolar bone and cementum are structures that provide support to the tooth. Any pathological process affecting periodontium is defined as periodontitis. For a long time, it was thought that gingivitis and periodontal disease appeared as a result of aging of the periodontal tissues that gave rise to inflammation and recession of the gingival tissues bone and finally

tooth loss. However, several studies have indicated that this is not just an adult disease, but also appears frequently in children (Escudero et al., 2008).

Gingiva is part of the mucosa of the oral cavity that covers the alveolar bone and serves to protect the underlying tissue. Normal gingiva has a pink color, a supple consistency and a stippling texture or orange peel. Periodontal ligaments are the connective tissues that surround the teeth and bind them to the bone. Periodontal ligaments serve to protect blood vessels and nerves, tooth attachment to bone and hard impact resistance due to occlusal stress. Alveolar bone is a hard tissue composed of layers of bone that serves as a support for teeth. The cementum is the part that envelops the tooth root, is hard, has no vena and serves as a periodontal ligament adhesion (Carranza et al., 2006).

Periodontitis comes from interactions between certain sub-gingival microorganisms, inflammation and immune responses. Aggressive periodontitis is predominantly the bacterium A.actinomycetemcomitans which is the cause of periodontal disease with progressive damage. Bacteria A.actinomycetemcomitans release several virulence factors as endotoxins and leukotoxins, and infection factors by localized and systemic humoral immune response (Carranza et al., 2002).

Bacteria A.actinomycetemcomitans have a number of virulence factors that help the progression of disease. Virulence determines the strength of the pathogenic potential and also means the relative capacity (quantity and quality) of the bacteria that cause damage to the host and its ability to control the body's defenses. Such bacterial virulence includes its capacity in tissue destruction, invasive bacterial levels, and the ability to avoid host defense responses (Carranza et al., 2006).

Bacteria present in the plaque, including lipopolysaccharide (LPS) and lipoteichoic acid, interact with toll-like receptors in epithelial cells, macrophages, leucocytes and fibroblasts, stimulating the production of cytokines such as TNF-α, IL-1β, IL-6, IL-8 and prostaglandin E2 (PGE2). To facilitate leukocyte infiltration, fibroblasts stimulated by TNF-α and IL-1β secrete matrix metalloproteinase (MMPs), which degrade extracellular matrix molecules including collagen. The inflammatory response of periodontal tissue can lead to tissue destruction and alveolar bone resorption (Suvan et al., 2011).

Tumor necrosis factor-α (TNF-α) plays an important role in periodontal inflammation. TNF-α is primarily produced by activated macrophages. TNF-α has a strong potential factor to increase bone resorption and is involved in degradation of connective tissue by stimulating PGE2 and collagenase (Moore et al., 1994).

The complex network of pro- and anti-inflammatory cytokines works on inflammatory periodontal tissues. Among other cytokines, interleukin-10 (IL-10) is an important multifunctional cytokine. Increased or decreased levels of IL-10 host are essential for balance control between inflamed individuals (Gray, 2000).

Interleukin-10 is an anti-inflammatory cytokine, produced by T-helper2 (Th2) cells, macrophages and B cells, which inhibit the synthesis of pro-inflammatory cytokines such as TNF-α, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), and interferon-γ (IFNγ). IL-10 suppresses the production of metalloproteinase, while increasing the synthesis of metalloproteinase inhibitors in macrophages. In addition, it stimulates the production of osteoprotegrin, which consequently inhibits bone resorption by preventing the involvement of the Receptor Activator of Nuclear Factor Kappa-B Ligand (RANK-RANKL). The IL-10 cytokine can be a protective cytokine in periodontal disease and regulate pro-inflammatory cytokines, including those involved in alveolar bone loss. Individuals who are high IL-10 level producers are more protected from periodontitis due to the anti-inflammatory role of IL-10. Therefore, elevated anti-inflammatory cytokine IL-10, will play a role in regulating immune response against periodontopatogenic bacteria (Bage, 2013).

The pathogenesis of periodontitis is initiated by bacteria that release LPS. LPS then activates inflammatory cells, resulting in the release of cytokines and local factors. At the same time, the bacterial components and inflammatory mediators react directly to the osteoblast or progenitor, resulting in a decrease in osteoblast function, and then the loss of adhesions of periodontal and dental tissue, including the alveolar bone and connective tissue. Periodontitis is an inflammation that extends through the gingiva and causes tissue damage through tooth attachment. The dominant bacteria in periodontitis are the gram negative ones that release the LPS by activating the host mechanism primarily that causes bone damage in periodontitis. Significant periodontal damage is clinically known as aggressive periodontitis (Carranza et al., 2006).

2 BACKGROUND

Aggressive periodontitis generally affects systemically healthy individuals less than 30 years of age, though patients may be older. Aggressive periodontitis is distinguished from chronic periodontitis by the age of onset, the rapid rate of destruction, composition of the subgingival microflora, alteration in the host immune response, familial aggregation of diseased individuals, and a strong racial influence (Joshipura, 2015).

Disease of the periodontium occurring in an otherwise healthy adolescent is characterized by rapid loss of alveolar bone about more than one tooth of the permanent dentition. The amount of destruction is not commensurate with the amount of local irritants (Albandar, 2014).

Key diagnostic criteria of this disease include an:

- Early age of onset, involvement of multiple teeth with a distinctive pattern of clinical attachment loss and radiographic bone loss.
- A relatively high rate of disease progression and the absence of systemic diseases that compromise the host's response to infection.
- Although in some patients the disease may start before puberty, in most patients the age of onset is during, or somewhat after, the circumpubertal period. A typical patient shows disease onset at an early age (i.e., before 25 years of age), although identification of the affected patient usually occurs after disease commencement.
- Initially, the periodontal lesions show a distinctive pattern, depicted radiographically as vertical bone loss at the proximal surfaces of posterior teeth, and the bone loss usually occurs bilaterally. In advanced cases of aggressive periodontitis the periodontal lesions may be depicted radiographically as a horizontal loss of bone. The primary teeth may also be affected, although early exfoliation of these teeth is not common.
- Aggressive periodontitis may be localized or generalized. In localized aggressive periodontitis (LAP), tissue loss usually starts at the permanent first molars and incisors, and with increasing patient age the disease may progress to involve the adjacent teeth. The generalized form of aggressive periodontitis involves most or all of the permanent teeth.

A.actinomycetemcomitans is a perio-pathogenic bacteria that has long been associated with localized aggressive periodontitis. The mechanisms of its pathogenicity have been studied in humans and pre-clinical experimental models. Although different serotypes of A. actinomycetemcomitans have differential virulence factor expression, A.actinomycetemcomitans cytolethal distending toxin (CDT), leukotoxin, and lipopolysaccharide (LPS) have been most extensively studied in the context of modulating the host immune response. Following colonization and attachment in the oral cavity, A.actinomycetemcomitans employs CDT, leukotoxin, and LPS to evade host innate defense mechanisms and drive a pathophysiologic inflammatory response. This supra-physiologic immune response state perturbs normal periodontal tissue remodeling/turnover and ultimately has catabolic effects on periodontal tissue homeostasis (Herbert, 2016).
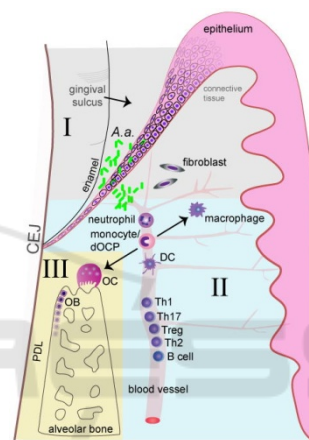

Figure 1 : A.actinomycetemcomitans colonizes the gingival sulcus by attachment to the sulcular/junctional epithelium cells (Herbert et al., 2016).

It subsequently invades through the epithelium via pro-apoptotic virulence mechanisms and penetrates into the subgingival connective tissue where is stimulates epithelial cells and fibroblasts to secrete pro-inflammatory cytokines (I). Neutrophils and monocytes are thereby recruited to the local site of infection and perpetuate the host inflammatory response. Subsequently, B and T cells are recruited to the diseased periodontium from the circulation (II). T cells secrete pro-resorptive factors that drive osteoclast (OC) formation and drive bone resorption. A.actinomycetemcomitans simultaneously impairs osteoblast (OB) function, perturbing bone remodeling processes, which ultimately results in catabolic alveolar bone loss (III).

A.actinomycetemcomitans virulence factors interact with host cells to initiate an aberrant inflammatory response in the periodontal gingival tissues. While it has been reported that trans-epithelial migration of polymorphonuclear

leukocytes (PMNs) into the gingival sulcus results in a formed pseudo-barrier, which is several cell layers thick between the plaque and junctional/sulcular epithelium surface (Garant, 1976), this review considers the gingival epithelium to be the initial barrier to A.actinomycetemcomitans. First responders are non-hematopoietic resident cells: gingival fibroblasts and epithelium cells. A.actinomycetemcomitans stimulates the host responses via exotoxic and endotoxic virulence factors, activating superficial epithelial cells and underlying fibroblast cells. A.actinomycetemcomitans can effectively migrate through the gingival epithelium and once A.actinomycetemcomitans bypasses these initial barriers, a host inflammatory response is initiated. Once A.actinomycetemcomitans penetrates deeper in the subgingival tissues, a broader host immune response is activated (Fives-Taylor et al., 1999).

A.actinomycetemcomitans immune stimulation in the periodontal microenvironment elicits a pathophysiologic pro-inflammatory state, which disrupts normal periodontal tissue remodeling processes ultimately promoting collateral tissue damage. In periodontal disease, the supra-physiologic level of pro-inflammatory and pro-resorptive cytokines favors alveolar bone resorption by monocyte or defined osteoclast progenitor (dOCP) derived osteoclasts, versus alveolar bone formation by mesenchymal derived osteoblastic cells. When bone resorption exceeds bone formation, an unbalanced bone remodeling process having catabolic effects on alveolar bone homeostasis, ultimately results in net alveolar bone loss (Baron et al, 1978). Bone-lining tartrate resistant acid phosphatase (TRAP) positive multinucleated osteoclasts secrete bone degradation enzymes, including matrix metalloproteinases (MMPs) and cathepsin K in the acidic sealing zone microenvironment, via integrin protein adherence to the bone surface (McCauley et al, 2002; Teitelbaum et al, 1997).

A.actinomycetemcomitans has been shown to induce osteoclast formation and bone loss in rodent animal models. Pro-inflammatory cytokines with potent pro-resorptive actions, including tumor necrosis factor (TNF)-α, IL-1, and IL-6 are highly upregulated by A.actinomycetemcomitans and thus promote osteoclast formation and bone resorption (Hotokezaka et al., 2007). In humans, A.actinomycetemcomitans positive patients had significantly greater periodontal bone loss than the A.actinomycetemcomitans negative subjects, supporting A.actinomycetemcomitans's remarkable

impact on periodontal disease associated alveolar bone loss (Fine et al., 2007).

Tumor necrosis factor-a (TNF-α) plays a role in periodontal inflammation. TNF-α is mainly produced by activated macrophages. TNF-α has strong potential to increase bone resorption and be involved in tissue degradation with prostaglandin-E2 and collagenase (Morimoto et al., 2008). Several studies have reported that there is an increase in TNF-α levels in crevicular gingival fluid (CGF) and gingival tissue in patients with periodontitis (Peggie et al., 2015). Pro- and anti-inflammatory cytokine tissue works on periodontal tissues that experience inflammation. Among other cytokines, interleukin-10 (IL-10) is an important multifunctional cytokine. Increased or decreased levels of IL-10 host are very important for controlling balance between individuals who experience inflammation (Gonzales et al., 2002).

Interleukin-10 is an anti-inflammatory cytokine, produced by T-helper 2 (Th2) cells, macrophages and B cells, which inhibit the synthesis of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-a (TNF-α) and interferon-γ (IFNγ). IL-10 production of radio metalloproteinase also increases the inhibitory of metalloproteinase tissue in macrophages (Lacraz et al., 1995). In addition, production of the osteoprotegrin hormone is a result of bone resorption by preventing the Kappa-B Ligan Factor Nuclear Receiver Activator (RANK-RANKL). Cytokines IL-10 can be protective cytokines in periodontal disease and regulate pro-inflammatory cytokines, including those involved in lost alveolar bone. Individuals who are high IL-10 producers are more protected from periodontitis due to IL-10 anti-inflammatory. Therefore, an increase in IL-10 anti-inflammatory cytokines will play an immune response to periodontopathogenic bacteria.

3 METHODS

This research was a true experimental study with Post Test Only Control Group Design. Rats were divided into 4 groups for 0.25; 0.5 and 0.75 CFU/mL, and negative control. Each group contained 5 rats. How to obtain A.actinomycetemcomitans bacteria with diluted culture using 700 μl PBS with 2% Sodium Carboxymethyl cellulose. Aggressive periodontitis in rats was induced by injecting A.actinomycetemcomitans, at 48 hrs and 96 hrs, post

injection the inflamatory signs were observed, three days later. Blood was centrifuged at 3000 rpm for 15 minutes to separate blood cells and plasma. Plasma is stored in a freezer of -80ºC then examination of TNF-α and IL-10 levels. Plasma were then collected to measure TNF-α dan IL-10 level by ELISA.

After obtaining data from the examination results, the data is processed in several stages. The data obtained is collected and checked whether there are writing errors, results mismatches, etc. so that it must be corrected. Data that has passed the editing process is coded so that it is easily displayed in the results table and it is analyzed. The data that has been coded is displayed in the table of the results of the examination so that it is easy to analyze and interpret its meaning. After tabulation, the data were analyzed according to the research objectives. In this study, the results of TNF-α and IL-10 ELISA test data from the patient's plasma were associated with the degree of blood of patients with normal control of plasma TNF-α and IL-10 ELISA test results. The relationship data for each observed group is described as looking for its meaning. Then the ELISA test result will be analyzed using ANOVA.

4 RESULT

Analysis of Variance (ANOVA) showed significantly increased levels of TNF-α in the infected group compared with that of the control group. Aggressive periodontitis in rats showed by redness, abscess and tooth mobility. This condition indicated that A. actinomycetemcomitans has the ability to adhere and invade the periodontal tissue further producing a colony that caused periodontitis.

Table 1. The mean and standard deviation of TNF-α levels in rats (Rattus norvegicus) with localised aggressive periodontitis.

Sample	Mean ± SD	Median	Max-Min	P
Control	777,4 ± 164,6	784,7	961,1-531	
P1 (Bakteri *Aa* 0,25 CFU/ml)	890 ± 41,84	886,9	948,7-832,7	0,0075
P2 (Bakteri *Aa* 0,5 CFU/ml)	1043 ± 67,24	1032	1137-976,9	
P3 (Bakteri *Aa* 0,75 CFU/ml)	1213 ± 96,87	1178	1347-1116	

Table 2. The mean and standard deviation of IL-10 levels in rats (Rattus norvegicus) with localised aggressive periodontitis.

Sample	Mean ± SD	Median	Max-Min	P
Control	576 ± 327	628,3	953,3-224,2	
P1 (Bakteri *Aa* 0,25 CFU/ml)	391,8 ± 135,9	402,7	556,1-178,8	0,0051
P2 (Bakteri *Aa* 0,5 CFU/ml)	125,4 ± 55,8	137	187,5-37,7	
P3 (Bakteri *Aa* 0,75 CFU/ml)	198,3 ± 52,1	224,2	248,5-137,1	

Table 3. Ratio between TNF-α levels and IL-10

	Mean		Ratio
	TNF-α	IL-10	
Control	777,4	576	1,3
0,25 CFU/ml	890	391,8	2,3
0,5 CFU/ml	1043	125,4	8,3
0,75 CFU/ml	1213	198,3	6,3

The highest ratio was found in the sample with the treatment of injection of A.actinomycetemcomitans bacteria with a concentration of 0.5 CFU / ml because it had the lowest IL-10 levels compared to the control group (K), P1 and P3.

5 DISCUSSION

In periodontal health the oral cavity is colonized by the oral commensal (non-pathogenic) flora. Under physiological states the normal oral flora stimulates the innate immune defense system in the periodontium, which controls bacterial colonization of periodontal tissues in close proximity to the gingival sulcus. In this periodontal microenvironment, A.actinomycetemcomitans infection induces a supra-physiological immune inflammatory response state, which disrupts normal periodontal tissue homeostasis in the gingiva, periodontal ligament (PDL), cementum, and alveolar bone, ultimately promoting tooth loss. A.actinomycetemcomitans virulence factors interact with host cells to initiate an aberrant inflammatory response in the periodontal gingival tissues. While it

has been reported that trans-epithelial migration of polymorphonuclear leukocytes (PMNs) into the gingival sulcus results in a formed pseudo-barrier, which is several cell layers thick between the plaque and junctional/sulcular epithelium surface (Garant, 1976). A.actinomycetemcomitans stimulates the host responses via exotoxic and endotoxic virulence factors, activating superficial epithelial cells and underlying fibroblast cells. A.actinomycetemcomitans can effectively migrate through the gingival epithelium and once A.actinomycetemcomitans bypasses these initial barriers, a host inflammatory response is initiated. Once A.actinomycetemcomitans penetrates deeper in the subgingival tissues, a broader host immune response is activated (Ahmed et al., 2001).
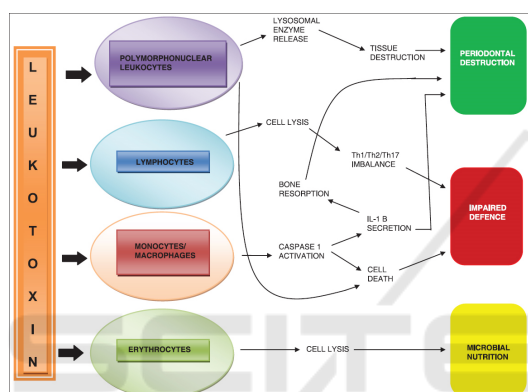

Figure 2. Effect of A.actinomycetemcomitans on human blood cells causing periodontal inflammation and tissue destruction (Malik et al., 2015).

One of the most studied virulence factors of A.actinomycetemcomitans is leukotoxin. This toxin is a 116 kDa protein produced by 56% of strains isolated from LJP patients. The mechanism of virulence leukotoxin is not only species specific but also cell specific. The toxin binds to neutrophils, monocytes, and a subset of lymphocytes; and forms pores in the membranes of these target cells overwhelming their ability to sustain osmotic homeostasis, resulting in cell death. Interaction is with polymorphonuclear leukocytes (PMNs). Leukotoxin has shown to efficiently cause death of human PMNs through extra-cellular release of proteolytic enzymes from both primary and secondary granules, along with activation and release of matrix metalloproteinase-8, which can contribute to periodontal tissue destruction (Malik et al., 2015).

The ability of leukotoxin to induce apoptosis in lymphocytes might impair the acquired immune response of periodontal infections. A shift in the balance between Th-1 and Th-2 subsets of T-cells is found in periodontal inflammation, with the Th-2 cells to associate with chronic periodontitis. Its ability to affect the lymphocytes also indicates a possible role of this molecule in Th-1/Th-2/Th-17 differentiation, important in inflammatory pathogenesis. Leukotoxin causes the activation of caspase-1, which is a cytosolic cysteine proteinase that specifically induces activation and secretion of the pro-inflammatory cytokines interleukin-1 and 18, which result in monocyte/macrophage lysis by incorporation in a cytosolic multimer complex named the inflammasome (Malik et al., 2015).

TNF-α is a strong pro-inflammatory cytokine, secreted by mononuclear leukocytes or macrophages in the early stages of the inflammatory response. TNF-α can stimulate osteoclasts, which lead to alveolar bone resorption, and can increase the release of matrix metalloproteinases (MMPs), which leads to the destruction of the extracellular matrix of periodontal tissue (Alexander et al., 1994). TNF-α is strongly associated with the pathogenesis and severity of periodontitis (Graves et al., 2003). Compared with healthy periodontal, there was an increase in TNF-α levels in Gingival crevicular fluid (GCF) periodontitis and in inflammatory periodontal tissues (Kennedy et al., 1990).

TNF-α affects cell migration by inducing and regulating adhesion molecules to encourage neutrophil turnover and adhesion to the vessel wall, which can cause extravasation. It also stimulates chemokine production, which is involved in the migration of infected cells and inflammation (Wajant et al., 2003). TNF-α also correlates with extracellular matrix degradation and bone resorption through the secretion of MMPs and RANKL (Graves et al., 2008).

Periodontitis is a disease that involves bone damage that can cause tooth loss. Therefore, anti-inflammatory cytokines are needed to inhibit bone resorption and increase alveolar bone regeneration which is also associated with the role of IL-10 in bone remodeling inhibiting bone resorption as well as reducing inflammation (Cochran, 2008). IL-10 is an anti-inflammatory cytokine that suppresses the immunoproliferative and inflammatory responses. As a factor produced by T helper2 cells (Th2), IL-10 inhibits cytokine production by Th1 cells. It is known that IL-10 is also produced by many other cell types, including B cells, mast cells, eosinophils, macrophages, and dendritic cells (DC), and a large number of T cell subsets such as CD8^{+} T cells and CD4^{+} T-regulation cells (Petska, 2004).

IL-10 can reduce the synthesis of pro-inflammatory cytokines and chemokines, such as IL-

1, IL-6, and TNF-α. This can also reduce the synthesis of nitric oxide, gelatinase, and collagenase. IL-10 succeeded in increasing the neutralization of synthesis of IL-1 and TNF-α. Therefore, IL-10 is also considered an important regulator of periodontal tissue homeostasis, in homeostatic and inflammatory conditions (Lee et al., 2009). IL-10 can directly inhibit osteoclast formation. The inhibitory effect of IL-10 on osteoclast formation is by direct action on osteoclast precursors. The molecular mechanism of this inhibition shows that IL-10 increases the expression of osteoprotegerin (OPG) but decreases the expression of NF-κB ligand receptor activator (RANKL) and colony stimulating factor-1 (CSF-1) (Liu et al., 2006).

Bone resorption is largely induced by the production of pro-inflammatory cytokines, such as TNF-a and IL-1. These cytokines can act by directly increasing the proliferation and activity of cells in osteoclasts or indirectly affecting the production of osteoclast differentiation factors such as RANKL and OPG through osteoblasts or stroma cells. IL-10 has been recognized to have strong anti-inflammatory activity for a long time, and this has proven to be an important endogenous suppressor of bone resorption which means IL-10 can suppress osteoclastic differentiation through the above aspects (Boyle et al., 2003).

Gingival epithelial invasion and apoptosis. In the in vitro model, A.actinomycetemcomitans was able to migrate through the gingival layer and epithelial cells by increasing the production of pro-inflammatory cytokines TNF-a, IL-1β, IL-6, IL-8 and increasing cell apoptosis (Dickinson et al., 2011). A study using A.actinomycetemcomitans in model mice found that apoptosis of A.actinomycetemcomitans was mediated by gingival epithelial cells via the caspase3 and caspase7 pathways (Kang et al., 2012). At the subcellular level, A.actinomycetemcomitans stimulates phosphorylation via TGFβR1 which signals gingival epithelial cells causing caspase3 activity to divide and subsequent cell apoptosis (Yoshimoto et al., 2014). This finding shows that A. actinomycetemcomitans bacteria have the potential to cross the gingival epithelium through the mechanism of pro-apoptosis. Disruption of gingival epithelial results in the induction of a periodontal pathological inflammatory microenvironment that supports recruitment of hematopoietic immune response cells derived to subgingival tissues (Yoshimoto et al., 2014).

Macrophages have been shown to play a role in the pathogenesis of periodontitis caused by A.actinomycetemcomitans. Monocytes enter the tissue which results in local infection with diapedesis from circulation where it can differentiate into activated macrophages or osteoclasts. Llike receptor toll receptors (TLRs) recognize pathogenic constituents and have been extensively studied in macrophage function. A.actinomycetemcomitans has strong endotoxic LPS received by TLR2, TLR4, and TLR5, with TLR4 currently being considered as the main receptor for LPS, which clearly illustrates the function of TLR2 and TLR4 signaling in macrophage interactions with A.actinomycetemcomitans (Park et al., 2014).

When bone marrow macrophages and TLR2 and TLR4 were stimulated with A.actinomycetemcomitans, TLR2 and TLR4 macrophages were attenuated and showed pro-inflammatory production of TNF-α and IL-6 cytokines. MyD88 is an adapter protein for all TLRs (except for TLR3), IL-1R, and IL-18R. MyD88 deficiency further reduces cytokine production by A.actinomycetemcomitans, suggesting that while TLR2 and TLR4 are critical regulators of A.actinomycetemcomitans, which induce the production of inflammatory cytokines, other receptors also spread TNF-α and IL-6 production. A.actinomycetemcomitans also stimulates the production of IL-12p40 (IL-12B) in macrophages, which mainly depend on MyD88. This finding clearly shows that TLR signaling is very important for the formation of inflammatory cytokines, but it has not been explained how TLR4 and TLR2 interact with A.actinomycetemcomitans to modulate their periopathogenesis (Park et al., 2014).

6 CONCLUSION

The induction of A.actinomycetemcomitans bacteria on periodontal tissues can cause periodontitis by being characterized when one of them is at high levels of TNF-α and low levels of IL-10 in rats (Rattus Norvegicus). In this case if TNF-α levels rise and IL-10 levels fall, the severity of infection and the prognosis of periodontitis in rats (Rattus Norvegicus) is getting worse.

High plasma level of TNF was seen in rats infected with 0.75 CFU/mL OF ac, while IL-10 was low as seen in rats infected with 0.5 CFU/mL OF Ac.

REFERENCES

Albandar, J., DeNardin, A., Adesanya, M., Diehl S., Winn D. 2001. *Associations between serum antibody levels to periodontal pathogens and early-onset periodontitis.* J Periodontol ;72:1463-9.

Baron, R., Saffar, J. 1978. *A quantitative study of bone remodeling during experimental periodontal disease in the golden hamster.* J Periodontal Res ;13:309–315

Brown, L., Brunelle, J, Kingman, A., 1996. *Periodontal status in the United States, 1988-1991: prevalence, extent, and demographic variation.* J. Dental Res. 75:672-683.

Escudero, N., Perea, M., Bascones, A., 2008. *Revisión de la periodontitis cronica: Evolución y su aplicacion. Avances en Periodoncia e Implantología Oral* 20(1):27-37.

Fine, D., Markowitz, K., Furgang, D. 2007. *Aggregatibacter actinomycetemcomitans and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents.* J Clin Microbiol ;45:3859–3869.

Fives-Taylor, P., Meyer, D., Mintz, K., Brissette, C. 1999. *Virulence factors of Actinobacillus actinomycetemcomitans.* Periodontology. ;20:136–167.

Graves, D. 2008. *Cytokines that promote periodontal tissue destruction.* J Periodontol 79: 1585–1591 .

Gray, J., 2000. *Parameter on Aggressive Periodontitis. Journal of Periodontology*, 71(5).

Herbert, B., Novince, C., Kirkwood, K. 2016. *Aggregatibacter actinomycetemcomitans, a potent immunoregulator of the periodontal host defense system and alveolar bone homeostasis.* NCBI Journal.

Joshipura, V., Yadalam, U., Brahmavar, B., 2015. *Aggresive Periodontitis.* India.

Lindberg TY, Bage T., 2013. *Inflammatory mediators in the pathogenesis of periodontitis.* Expert Rev Mol Med 15: e7

Liu, S. Yao, and G. E.Wise, 2006. *Effect of interleukin-10 on gene expression of osteoclastogenic regulatory molecules in the rat dental follicle.* European Journal of Oral Sciences, vol. 114, no. 1, pp. 42–49

Malik, R., Changela, R., Krishan, P., Gugnani, S and Bali, D. 2015. *Virulence factors of Aggregatibacter actinomycetemcomitans.* Journal of International Clinical Dental Research Organization. Vol 7

McCauley, L., Nohutcu, R., 2002. *Mediators of periodontal osseous destruction and remodeling: principles and implications for diagnosis and therapy.* J Periodontol ;73:1377–1391.

Moore WE, Moore LV., 1994. *The bacteria of periodontal diseases. Periodontology* 2000 5:66-77.

Newman MG, Takei HH, Carranza FA, 2002. *Clinical Periodontology. 9th ed. Philadelphia: WB Saunders.*

Newman M.G, Takei H.H, Klokkevoid P.R and Carranza F.A., 2006. *Carranza's Clinical Periodontology, 10th. St.Louis Missouri: Saunders Elsevier,*: p 46-7, 68, 72-75, 116-120.

Park, S., Kim, D., Han, S. 2014. *Diverse Toll-like receptors mediate cytokine production by* Fusobacterium nucleatum *and Aggregatibacter actinomycetemcomitans in macrophages.* Infect Immun ;82:1914–1920.

Pestka, C., Krause., Sarkar, D. 2004. *Interleukin-10 and related cytokines and receptors,* Annual Reviewof Immunology, vol. 22, pp. 929–979.

Suvan J, D'Aiuto F, Moles DR, Petrie A, Donos N., 2011. *Association between overweight/obesity and periodontitis in adults. A systematic review.* Obes. Rev. 12(5):e381-404.

Teitelbaum, S., Tondravi, M., Ross, F. 1997. *Osteoclasts, macrophages, and the molecular mechanisms of bone resorption.* J Leukoc Biol ;61:381–388.

Yamamoto M, Kobayashi R, Kono T, Bolerjack B, Gilbert R., 2011. *Induction of IL-10- producing CD4 T-cells in Chronic Periodontitis.* J. Dent. Res. 90(5):653-658.