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Chapter 3

Anaerobic Bacteria Associated with Periodontitis

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Additional information is available at the end of the chapter

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Abstract

Oral bacteria are highly associated with oral diseases, and periodontitis is a strongly prevalent disease, presenting a substantial economical burden. Furthermore, there is a strong association between periodontal bacteria and other diseases, such as cardiovascular disease, rheumatoid arthritis, or diabetes, so it becomes clear that efficient periodontal cure would be of good medical benefit to general health. Periodontally, Healthy loci show a low number of bacteria which are cultivable by individual sulcus, 10^2–10^3 microorganisms with almost Gram-positive microbiota, including *Streptococcus* and *Actinomyces* species. In gingivitis, it is characterized by an increased bacterial number, 104–105 microorganisms by periodontal sulcus, besides an increased diffusion of Gram negative bacteria (15–50%).The increased number of oral bacteria could be associated with the decreased role of the innate and adaptive immunity; so, this chapter will focus on the most prevalent bacteria associated with the oral disease on the one hand and the role of innate immunity and adaptive immunity (Interleukin 1 Beta Il-1β and Tumor necrosis factor-alpha TNF-α) in oral diseases on the other hand.

Keywords: anaerobic bacteria, oral bacteria, oral diseases, periodontitis, oral immunity

1. Introduction

Oral bacteria are highly associated with oral diseases; periodontitis is a strongly prevalent disease, presenting substantial economic problem [1]; and oral disease are associated with other diseases, such as cardiovascular, rheumatoid arthritis, or diabetes, so it becomes clear that good periodontal cure would be of excellent medical interest to general health [2]. Periodontally, healthy sites show a low number of bacteria which are cultivable by individual sulcus, 10^2–10^3 microorganisms with almost Gram-positive microbiota, including

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Streptococcus and *Actinomyces* species. In gingivitis, it is characterized by an increased bacterial number, 10^4–10^5 microorganisms by periodontal sulcus besides an increased diffusion of Gram-negative bacteria (15–50%) [3]. The increased number of oral bacteria could be associated with the decreased role of the innate and adaptive immunity; so, this chapter will focus on the most prevalent bacteria associated with the oral disease on the one hand and the role of innate immunity and adaptive immunity (interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α)) in oral diseases on the other hand.

2. Historical review on the classification and identification of oral bacteria

The initial date for the identification of oral bacteria belongs to 1680, when Antonie van Leeuwenhoek noticed, described, and isolated the microorganisms from his teeth plaque by using a primitive microscope. He drawn the noticed microbes and, when he established with the current knowledge, these drawings represented the most plentiful bacteria found within the oral cavity, including fusiform, spirochetes, and cocci bacteria [4].

Record research, a wide range of clinical studies on animals, engaged these oral bacteria with two common diseases, periodontitis, and dental caries. Even long before the visual observations of microorganisms, about 5000 BC, the Sumerians accused certain form of living (called as tooth worm) as a causative agent of caries on teeth [5]. Limited microbiological cultivation procedures and isolation techniques beginning of the nineteenth century forbid scientists to identify the exact causative agent of the disease. But this finding was partially done in 1925, by Clarke [6]. Unlike dental caries, another human oral disease is called periodontitis, and it is considered as the second most common disease worldwide. The early studies including oral bacteria in the pathogenesis of periodontitis were done on a hamster. Administration of penicillin inhibited-periodontitis in hamster gives a clear evidence of a bacterial agent [7]. Some studies isolated bacteria from dental caries, called *Streptococcus mutans* and described its ability to ferment many sugars and produce acids in glucose broth (pH of 4.3). However, he was not able to prove that *S. mutans* actually produces dental caries, but this finding was experimentally proven later in 1960 [8]. Whereas the infectious case of periodontitis appeared by demonstration of its transmissibility during infection from a person to another [9]. For a long time, periodontal disease researchers aimed to determine specific bacteria from a complex microbial plaque that may be considered a sole causative agent of periodontitis. The big problem was the cultivation of oral bacteria in laboratory. Most of the oral bacteria are anaerobic that died by air and considered fastidious microbes. This was recognized by researchers at that time. Major progress in the anaerobic culture was done in 1960 by designation of anaerobic glove boxes (a primitive form of now widely used anaerobic chambers), and it was used for the first time by Socransky [10]. This invention improved anaerobic cultivation techniques and was combined with optimized complex culture media; it allowed the invention of a pure and a good culture of more than 300 oral bacteria types in the period of 40 years ago, including clinical samples from supragingival and subgingival dental plaque taken from diseased and healthy subjects [11].

The studies on healthy subjects who agreed to take toothbrushing for a prolonged period appeared direct association between assembly of dental plaque and the initiation of gingiva diseases, mild form of oral diseases [12, 13]. After 28 days without basic oral hygiene in periodontally healthy subjects, there was a rapid assembly of bacterial plaque on the surface of teeth, and gingivitis was developed in all subjects within 10–21 days. These damages were reversible when toothbrushing was reintroduced. The researchers analyzed the smear of dental plaque specimen taken during the 28th day, and they found, at first, colonizing bacteria on the surface of the teeth, bacteria which belonged to the Gram-positive cocci and rods, Gram-negative cocci and rods, filaments, and fusobacteria, respectively, while finally spirochetes and spirilla were taken place in some times during colonizing. The outside of clinical gingivitis linked with the manifestation of the Gram-negative bacteria, and other studies on the microbial rotation in oral plaque formation confirmed these outcomes [14]. Through the years' progress, many other culture-based and molecular methods were given a huge information about the type of species included in periodontitis. A passionate dentist, W. D. Miller, studied hard for a long time in the of Robert Koch's laboratory trying to discover the microorganisms which were responsible for teeth decay; he published his research in 1980, with a book called *Microorganisms of the Human Mouth*; and in the same book, he suggested a chemoparasitic theory. According to that theory, in a sensitive host, carbohydrates fermentable oral microorganisms convert carbohydrates into acid, then the acid demineralizes tooth structure specially enamel [15, 16].

The classification of periodontal pathogens was tried to figure out by many researchers. The most understanding classification divided the periodontal pathogens into color-coded clusters published by Socransky and his team in 1998. This division resolves and identifies many problems and complexes of bacteria and clears their series of infection in the oral plaque and their role in periodontitis. Biofilm structure, which extends away from the tooth surface, was essential in this classification, and the bacteria responsible for dental plaque were classified into six clusters (red, orange, yellow, green, blue, and purple). *Actinomyces odontolyticus* and *Veillonella parvula* represented the "purple" form, while species of *Streptococci* including *S. sanguinis* and *S. oralis* refer to the "yellow" form [17].

The first colonizers of the surface of the teeth with *Actinomyces* species are purple and yellow form of this classification. The next complex, designated with green, included *Capnocytophaga* spp., *Campylobacter concisus*, *Eikenella corrodens*, and *Actinobacillus actinomycetemcomitans*, the bacteria contributing to the primary changes in the host. The "bridging species" formed the orange cluster are as follows: *Prevotella* spp., *Micromonas micros*, *Fusobacterium* spp., *Eubacterium* spp., and *Streptococcus constellatus*. That cluster included the species capable of using and secreting nutrients in the biofilm, in addition to expressing cell surface molecules facilitating binding to early colonizers, and the individual of the red complex. Finally, *P. gingivalis* and *T. denticola* in addition to *Tannerella forsythia* refer to the red cluster, and these are considered the prevalent pathogens in periodontitis progression; however, there is a clear association between the prevalence, number of these bacteria, and periodontitis clinical parameters [17, 18]. These three bacteria (in particular *P. gingivalis*), besides individuals of the orange cluster also linked with periodontal lesions, have been heavily studied in vitro, aiming to the identification of their key virulence mechanisms [18].

3. Most prevalent diseases caused by oral bacteria

Many major periopathogens can be seen in healthy individuals of all ages, indicating the coexistence of these bacteria as a normal flora in the host. These bacteria increase their numbers over time, and this change depends on the conditions of the internal or external environment, and it induces chronic periodontal inflammation that can cause the teeth loss as an outcome destroying the alveolar bone [19]. The inflammation of the tissues around the tooth due to accumulation of dental plaque is considered the main characteristic of acute and chronic periodontitis. The current classification of oral disease included the following [20]:

- Gingivitis: Plaque triggers inflammation in the gingivae that are characterized by red, swollen tissues and bleeding while brushing or probing.
- Chronic periodontitis: The connective tissue attachment of the teeth and destruction of junctional epithelium are damaged. Periodontal pockets and alveolar bone destruction occurred, and this state leads to chronic periodontitis.
- Aggressive periodontitis: It is a severe condition that represented the high proportion of younger cohort patients, the progression of disease is rapid, and the degree of destruction of the tissue (connective tissue) is high. The higher the level of the plaque, the higher the level of the disease.
- Necrotizing ulcerative gingivitis (NUG): Painful ulceration of the tips of the interdental papillae. Grey necrotic tissue is visible and there is an associated halitosis. The condition is termed necrotizing ulcerative periodontitis (NUP).
- Periodontal abscess: Inside the periodontal pocket is a different species of bacteria when the immune system responded to infection, and the periodontal abscess is form. Acute or chronic condition may occur, and in some time, the condition is asymptomatic.
- Perio-endo lesions: Lesions may be coalescing or independent, and the periodontal pathogen source originates either in the root canal system or in the periodontium.
- Gingival enlargement: The thickness occurs in response to irritation caused by plaque or calculus, and the other responses are repeated friction or trauma changes in hormone levels or in some time the effect of a drug.

The most common periodontopathogen correlated with aggressive forms of periodontitis is *Aggregatibacter* (previously *Actinobacillus*) *actinomycetemcomitans*. This small Gram-negative coccobacillus, capnophilic and non-motile have been determined as the most causative factor of aggressive periodontitis in young individuals and adults [21]. *A. actinomycetemcomitans* has been divided into six serotypes, and it has been postulated that some serotypes are correlated with periodontitis more frequently than periodontal health. Exemplifying this relationship, serotype C has appeared more repeatedly from healthy subjects and serotypes A and B more frequently in periodontitis [22]. But differences are pointed in *A. actinomycetemcomitans* serotype distribution when ethnicity and geographic location are taken into account; still, 3–8% of strains have remained nonserotypeable [23].

Gram-negative obligate anaerobe asaccharolytic bacteria (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) have been extensively correlated with periodontitis [17]. *P. gingivalis* has been detected in correlation with periodontal damages and has an arsenal of virulent factors that can affectively stimulate the host responses [18]. *T. forsythia* was first described at the Forsyth Institute, and it became a recognized periodontopathogen because of its repeated detection from sites with periodontitis and its huge correlation with the formation of pocket with deep size [24]. *T. denticola* is also frequently presented in periodontitis subgingivally sites, and their number is decreased after appropriate treatment [25]. Other bacteria that have been related with periodontitis include *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *Selenomonas*, *Eubacteria*, *Eikenella corrodens*, *Campylobacter rectus*, and *Parvimonas micra* [26].

Molecular microbiological studies have shown that many of the bacteria species are recognized in correlation with periodontitis and expanded to include uncultivated and less-often-identified phylotypes [27].

4. Mechanisms of destruction in periodontal tissues

Bacteria can cause damage directly and indirectly. Various mechanisms are described in the steps below. Cytotoxic cellular immune responses to self- and pro-inflammatory responses involving release of interleukin-1 beta (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) could lead to tissue destruction [28].

- Crevicular epithelium is destroyed by Porphyromonas gingivalis, Treponema denticola, and Aggregatibacter actinomycetemcomitans.
- Leukotoxin is secreted by A. actinomycetemcomitans, and it is impaired with polymorphonuclear (PMN) function (chemotaxis, phagocytosis, and intracellular killing) and other leukocytes.
- P. gingivalis is dysregulated of cytokine networks by their R1 proteinase activity.
- Capnocytophaga spp. are degraded of immunoglobulins.
- P. gingivalis, *P. intermedia*, T. forsythia, and T. denticola increase the mucosal permeability and degradation of collagen by fibroblastic collagenase by volatile sulfur compounds from Gram-negative anaerobes in addition to disaggregation of proteoglycans by disrupting SH (sulphydryl) bonds or impaired host cell function.
- Destruction of periodontal tissues proteins by proteolytic enzymes (collagenases and trypsin-like proteinases) to peptides and amino acids provides nutrients for Gram-negative bacteria. While the extracellular matrix is destroyed by other type of enzymes that called hydrolytic enzymes.
- The complement is activated when infection occurs by bacteria in response to LPS.
- Lipoteichoic acid from Gram-positive bacterial cell walls stimulates bone resorption.

5. Immunopathological factor associated with periodontal pathogens

The pathogenesis of periodontal disease is categorized into four stages, based on histopathological examination of the development of periodontal inflammation due to plaque accumulation. These stages are called **(a)** the initial, **(b)** the early, **(c)** the established, and **(d)** the advanced lesions [28, 29]. The description of stages in periodontal damage progression is listed below:

(a) Initial lesion

Without normal oral hygiene measures, within 2–4 days of plaque accumulation, the first inflammatory response is observed histologically. It is characterized by vasodilatation, loss of perivascular collagen, and active migration of monocytes and neutrophils into the periodontal tissues and junctional epithelium mediated by endothelial leucocyte adhesion molecules (ELAM) and intercellular adhesion molecules (ICAM) that are observed. The exudation of serum proteins from the dilated capillaries leads to an increase in gingival crevicular fluid (GCF) flow.

(b) Early lesion

The early lesion presents after 4–7 days of plaque accumulation. This is clinically detectable as gingivitis, with more pronounced vascular changes and an increase in extravascular neutrophils. Histologically, the inflammatory infiltrate consists of numerous lymphocytes (predominantly T lymphocytes), immediately below the proliferating basal cells of the junctional epithelium. Destruction of the gingival connective tissue occurs through apoptosis of fibroblasts, and a reduction in the collagen fiber network of the marginal gingivae occurs via host- and pathogen-derived MMP.

(c) Established lesion

This is similar to the early lesion with a shift in the cell population in the inflammatory (2–3 weeks of plaque accumulation). Here, plasma cells are the main histological features in older patients, whereas in younger patients, the infiltrate continues to be dominated by lymphocytes. Clinically, inflammation will become more pronounced with an increase in swelling, and the false pocket will form. T and B lymphocytes, antibodies, and complement are found in the inflamed marginal gingival and gingival sulcus.

(d) Advanced lesion

At this stage the inflammatory lesion expands into the periodontal ligament and alveolar bone. There is a destruction of a tissue linked to the teeth. The junctional epithelium migrates down the root surface to form a true periodontal pocket. MMP has the ability to destroy periodontal ligament and the surrounding alveolar bone through enhanced osteolytic activity. The direct cytotoxicity of bacterial products leads to direct tissue damage. Proteinases, collagenases, epitheliotoxin, cytolethal distending toxin, hemolysin, hydrogen sulfide, and ammonia are examples of bacterial products. Moreover, dysregulation of the factor derived from the host such as proteinases and proteinase inhibitors; MMPs and tissue inhibitors to metalloproteinases (TIMPs); pro-inflammatory cytokines such as IL-1α, IL-1β, TNF-α, and

others; prostaglandins; and the products of polymorphonuclear leukocytes leads to the damage of the connective tissue attachment.

5.1. Innate immunity response to periodontal pathogens

The innate host response primarily involves the recognition of microbial components such as LPS by the immune cells of the host, and the result of activation produced inflammatory mediators. The Toll-like receptors (TLRs), which are synthesized by leukocytes and resident cells in the periodontal tissues, can activate the innate immunity response by binding to numerous bacterial components [30–31]. The developing biofilm consists of initially Gram-positive cocci in health, changing to the increased numbers of motile Gram-negative anaerobes in gingivitis and periodontitis [17].

Endotoxin (LPS) of Gram-negative bacteria is considered a huge stimulator of TLR4. LPS from Gram-negative bacteria cell wall can be released through cell lysis. It becomes linked to the extracellular acute-phase protein LPS-binding protein before binding to the cluster of differentiation 14 (CD14). The outcome is transferred from LPS to the extracellular domain of the TLR4 receptor and subsequent TLR4 signaling [32]. Gram-negative bacteria also activate TLR2 through their cell membrane proteins, TLR5 through flagella, TLR9 through the determination of bacterial cytosine-phosphate-guanine (CpG) DNA, and nucleotide-binding oligomerization domain-containing proteins 1 and 2 (NOD 1, NOD 2) through peptidoglycan derivatives [32, 33].

Periodontal pathogens have been reported to stimulate TLRs in vitro, such as LPS of *P. gingivalis*, and fimbriae is a potent TLR2 agonists [34–36]. *A. actinomycetemcomitans* and whole *P. gingivalis* will stimulate TLRs [37–40]. Moreover, many bacteria can initiate an immune response via TLR9, which also detects viable bacterial DNA [41]. It is therefore clear that the myriad of bacteria that are found in both health and increasing hardness of periodontitis will present a challenge to the response innate immunity. Following TLR activation, an intracellular signaling cascade occurs which can result in stimulation of transcription factors, subsequent inflammatory cytokine expression, leukocyte migration to the infection locus, and tissue damaging [42, 43]. The nucleotide-binding oligomerization domain (NOD) and the inflammation system have been submitted as possible accessory molecules in the induction of response of innate immunity against periodontopathogens [44–46]. The junctional epithelium is the front line between the oral normal flora and the host. It is well equipped to recognize invading pathogens, some studies showed that the present of mRNA encoding TLR2, TLR3, TLR4, TLR5, TLR6, and TLR9 in gingival epithelial cells is a clear indication of the existence of the infectious agent [47]. Within the gingival epithelium and between the connective tissue, Langerhans cells and tissue dendritic cells are also found. TLRs are produced by antigen-presenting cells and appear on their surface including TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR8, and TLR10. The response of adaptive immunity against bacterial products is monitored by these receptors [30, 33].

The alveolar bone is the supporting structure into which the periodontal ligament inserts that is ultimately destroyed by the inflammatory lesion of periodontitis. Osteoblasts and osteoclasts included in bone turnover also express TLR1, TLR4, TLR5, TLR6, and TLR9 [35] and TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9, respectively [48]. It is therefore possible that TLR signaling within the bone can generate an inflammatory response to invading pathogens,

leading to pathological resorption of the bone through excessive or prolonged production of osteolytic host molecules, including IL-1, tumor necrosis factor-α (TNF-α), and prostaglandin E2 (PGE2), which stimulate osteoblast inhibition and osteoclast activation and maturation through the receptor activator of nuclear factor kappa-B ligand/osteoprotegerin (RANKL/OPG). Many biological events in periodontal disease are obligatory regulated by cell–cell interactions, which may be grouped into two forms: cognate (adhesive) interaction, achieved by mutual recognition between membrane-bound cell surface molecules, and cytokine-mediated interactions [49].

Intercellular adhesion molecule-1 (ICAM- 1, CD54) and ITGB2 (integrin beta 2, CD18), which stabilize cell–cell interactions and facilitation of leukocyte migration across the endothelial barrier, are achieved by ICAM-1 (intercellular adhesion molecule-1, CD54) and ITGB2 (integrin beta 2, CD18); therefore, they are called adhesion molecules [22].

5.1.1. Adaptive immunity cytokine (pro-inflammatory cytokines) response to periodontal pathogens

Cytokines are a large and diverse family of soluble mediators including interleukins. Cytokines play a major role in various biological activities such as differentiation, proliferation, regeneration, development, repair inflammation, and homeostasis. Cytokine networks are an important side of periodontal inflammation and subject to several excellent reviews [50].

The IL-1 family of cytokines (IL-1α and IL-1β) has different roles in immunity, tissue homeostasis, tissue breakdown, and inflammation. Monocytes and macrophages are released TNF-α in huge amount in responses for infection. It induces the production of collagenase and is secreted by fibroblasts to make damages on the cartilage and bone, and it has been involved in the damage of the periodontal tissue in periodontitis [51].

5.1.2. Interleukin-1α and interleukin-1β (IL-1α/IL-1β) role in periodontal pathogens

IL-1 is a polypeptide, which has diverse activities and roles in immunity, inflammation, tissue breakdown, and tissue homeostasis [52]. IL-1 is synthesized by various cell types, such as fibroblasts, lymphocytes, skin cells, macrophages, monocytes, vascular cells, and osteocytes, following its activation. IL-1α and IL-1β belong to the IL-1 family of cytokines which have similar biological functions and bind to the same receptors found on many cell types. Fibroblast cells in periodontal ligament are triggered by IL-1 to stimulate them to release cellular mediators, prostaglandin E2 (PGE2), and matrix-degrading enzymes which destroyed the connective tissue and lead to attachment loss [53]. Some studies refer that IL-1 is involved in the pathogenesis of periodontitis and also associated with bone destruction. Together, IL-1α and IL-1β have appeared to stimulate bone resorption and bone inhibition in cooperation with TNF-α. IL-1β has appeared to be significantly more potent in mediating bone resorption compared with IL-1α and TNF-α. IL-1 can also stimulate elevated production of matrix metalloproteinases (MMPs), procollagenase, and plasminogen activator [54].

5.1.3. Tumor necrosis factor-alpha (TNF-α) role against periodontal pathogens

TNF-α is a pro-inflammatory cytokine released by activated monocytes and macrophages [55]. TNF-α functions include the upregulation of attachment molecules and chemokines which

are involved in the cell migration to inflamed and infected sites [56]. Collagenase secreted by fibroblasts, resorption of the cartilage and bone, and damaging of the periodontal tissue all are stimulated by cytokine production [57]. Both GCF and periodontitis tissues have shown high levels of TNF-α, and it has shown positive correlation to MMP and RANKL expression [58, 59]. Animal studies also demonstrated that TNF-α plays a key role in inflammation and periodontal tissue damaging including bone resorption and loss of connective tissue attachment [58, 60]. Pro-inflammatory cytokines produced during infection (IL-1β and IL-6) are upregulated by TNF-α, this production linked with cell migration into the site of infection, and finally bone resorption occurred [55, 61]. New studies was done by Alwaeli and Abd [62, 63] who tried to interpret the relation between concentration of TNF-α and IL-1β and polymorphism of their genes, and they found some of SNPs (single-nucleotide polymorphisms) that trigger the production of TNF-α and IL-1β, by increasing the activity of their genes so the high concentration level of TNF-α and IL-1β leads to additional damage in periodontal tissue, while the other SNPs decrease the production of TNF-α and IL-1β, for this reason the termed "SNP-genotype combination principal" for this phenomena by Alwaeli and Abd (62–63).

List of abbreviation

BC	Before Christ
CD	cluster of differentiation
CpG	cytosine-phosphate-guanine
ELAM	endothelial leukocyte adhesion molecules
GCF	gingival crevicular fluid
ICAM	intercellular adhesion molecules
IL-1β	interleukin-1 beta
ITGB2	integrin beta 2
LPS	lipopolysaccharide
MMP	matrix metalloproteinases
NOD	nucleotide-binding oligomerization domain
NUG	necrotizing ulcerative gingivitis
NUP	necrotizing ulcerative periodontitis
OPG	osteoprotegerin
PGE2	prostaglandin E2
PMN	polymorphonuclear
RANKL	receptor activator nuclear factor kappa-B ligand

SNP	single-nucleotide polymorphism
TIMP	tissue inhibitors to metalloproteinases
TLRs	toll-like receptors
TNF-α	tumor necrosis factor-alpha

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