Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

IMMUNE DEFENSE AND REPAIR MECHANISMS IN CARIES AND PERIODONTITIS

Madrid, academic year 2022/2023

Identification number: 139

SUMMARY AND KEY WORDS

Introduction: Caries and periodontitis are among the most common oral diseases. They have a multifactorial etiology where the bacterial composition of the dental plaque plays a key role. The different elements of the oral cavity such as saliva, microbiota, and tissue integrity play a role in maintaining a healthy environment to avoid the onset of disease. As soon as a threat is detected, oral immunity intervenes with the objective of returning to a healthy state; **Objectives**: To understand how the oral cavity acts to prevent and protect teeth and tissues from bacterial aggression and how it can be beneficial or detrimental for the host; **Methodology**: A systematic review was conducted based on the study of scientific articles from the last 15 years and found in different databases. Inclusion and exclusion criteria were established; **Results**: 29940 articles were found regarding the keywords used to perform the research. After removing duplicates, applying exclusion and inclusion criteria, and studying abstracts, 14 full-text articles were analyzed and organized in table form; **Conclusion**: The oral cavity is a complex medium that presents a multitude of defense mechanisms, including saliva function and flow, presence of symbiotic microbiota into the oral cavity, tissue integrity, and immunity. These mechanisms work in a symbiotic and balanced way to maintain a healthy environment and prevent the onset of caries or periodontitis. Each mechanism has independent and interconnected roles. The key to success in protecting the host is balance. The imbalance of one of these mechanisms, particularly immunological reactions, can lead to the progression or aggravation of a disease.

Keywords: Saliva; oral microbiota; caries; periodontitis; chemical mediators; cellular immunity; immune defense.

RESUMEN Y PALABRAS CLAVE

Introducción: Las caries y la periodontitis están entre las enfermedades orales más comunes. Tienen una etiología multifactorial donde la composición bacteriana de la placa dental juega un papel clave. Los diferentes elementos de la cavidad oral, como la saliva, la microbiota y la integridad del tejido, desempeñan un papel en el mantenimiento de un entorno saludable para evitar el inicio de la enfermedad. Tan pronto como se detecta una amenaza, la inmunidad oral interviene con el objetivo de volver a un estado saludable; **Objetivos:** Comprender cómo actúa la cavidad oral para prevenir y proteger los dientes y los tejidos de la agresión bacteriana y cómo puede ser beneficioso o perjudicial para el huésped; **Metodología:** Se realizó una revisión sistemática basada en el estudio de artículos científicos de los últimos 15 años y encontrados en diferentes bases de datos. Se establecieron criterios de inclusión y exclusión; **Resultados:** Se encontraron 29940 artículos con respecto a las palabras clave utilizadas para realizar la investigación. Después de eliminar duplicados, aplicar criterios de inclusión y exclusión y estudiar resúmenes, se analizaron y organizaron en forma de tabla 14 artículos completos; **Conclusión:** La cavidad oral es un medio complejo que presenta una multitud de mecanismos de defensa, incluyendo la función y el flujo de la saliva, la presencia de microbiota simbiótica en la cavidad oral, la integridad del tejido y la inmunidad. Estos mecanismos trabajan de manera simbiótica y equilibrada para mantener un entorno saludable y prevenir el inicio de caries o periodontitis. Cada mecanismo tiene roles independientes e interconectados. La clave del éxito para proteger al huésped es el equilibrio. El desequilibrio de uno de estos mecanismos, particularmente las reacciones inmunológicas, puede llevar a la progresión o agravamiento de una enfermedad.

Palabras clave: Saliva; microbiota oral; caries; periodontitis; mediadores químicos; inmunidad celular; defensa inmunológica.

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1 INTRODUCTION

The most common bacterial infections that affect people include periodontitis and dental caries. They appear after the accumulation of pathogenic dental plaque (1–3). These diseases have multifactorial and polymicrobial etiologies that are influenced by cooperative interactions between the host immune system and various microorganisms, as well as other host variables and environmental inputs (2).

The host's innate immunity and protective mechanisms are crucial for defending against pathogens in the oral bacterial biofilm. As the oral tissues are constantly exposed to various triggers, they must act swiftly to prevent infection in both hard and soft tissues. (1,4).

Periodontal diseases and caries are characterized by a complex and coordinated host response system that has the role of protecting the host with the least amount of collateral harm to host cells and tissues (5).

Host defense mechanisms may also trigger tissue repair processes in certain cases. (1).

1.1 Caries

One of the most common chronic disorders affecting people worldwide is dental caries (6). It is a multifactorial and dynamic disease caused by dietary sugar-driven bacterial growth and metabolism, disrupting dental mineralization homeostasis, and leading to localized acidity. (2,7,8). Caries are chronic endogenous infections that progress slowly in most individuals (9).

Caries is the most common disease in the world and the most common cause of tooth loss and pain (8,10).

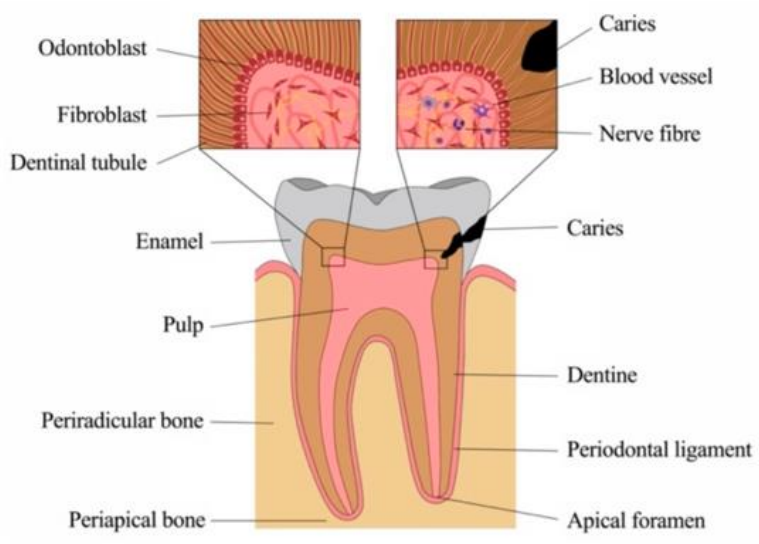

Figure 1: illustration of tooth composition with a caries lesion (11).

1.1.1 A multifactorial disease

Initiation of caries lesion depend on three main factors: acidogenic and acidophilic bacteria, the presence of carbohydrates from the diet and host factors (8).

Oral biofilm alone cannot cause dental caries. Dental plaque, composed of bacteria, can cause the disease only when other factors are present. When cariogenic microorganisms are minor and demineralization and remineralization are balanced, homeostasis is maintained (10).

Numerous changes in the oral microbiome occur because of genetic and environmental influences (10). Bacterial fermentation of the food waste, which is the main factor responsible for the changes in microbiota, produces acids that decalcify hard tissues (enamel, dentin, and cement) and allow for the decay to progress until the pulp (7,11,12). The oral microbiome and immune system, which are impacted by environmental and genetic factors, drive each person's unique caries risk (10,13).

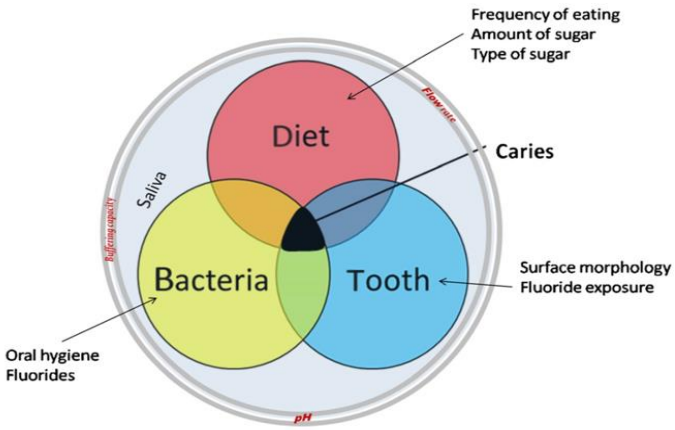

Figure 2: main factors influencing the apparition of caries (14).

1.1.2 Caries development

This process begins with the appearance of dental plaque, which is a layer of germs that coats the tooth surface, facilitated by the non-shedding properties of the tooth surface (3,7,15). Where oral biofilms are allowed to build up and remain on teeth for extended periods of time, caries lesions form (7). Frequent consumption of fermentable carbohydrates leads to low plaque pH, disrupting microbial balance, and causing demineralization. Long-term low pH in biofilm enhances bacterial acidogenicity and acidurance (9,10).

This series of events may cause a change in structure: the enamel becomes rough, and the dentin becomes soft (9).

1.1.3 Diagnostic and classification of caries

Controlling the progression of caries presents the most difficulty in the management of the caries process. Finding early non-cavitated caries lesions and monitoring their activity are crucial if we wish to control it (10).

The most used technique to diagnose caries is the combination of visual and tactile diagnosis as well as bitewing radiographies (6). Nowadays, other non-invasive methods and tools are also useful for caries detection particularly at an

early stage, such as Quantitative Light-induced Fluorescence, DIAGNOdent, Fibre-optic Transillumination and Electrical Conductance (6,13).

Caries can be classified by their location (coronal or root/cementum surface), severity (non-cavitated, cavitated), depth (enamel, dentin, pulp), and activity (active or inactive) (13).

ICDAS, implemented in 2001, classifies caries severity via visual and tactile examination using a ball-ended explorer on dry teeth (6).

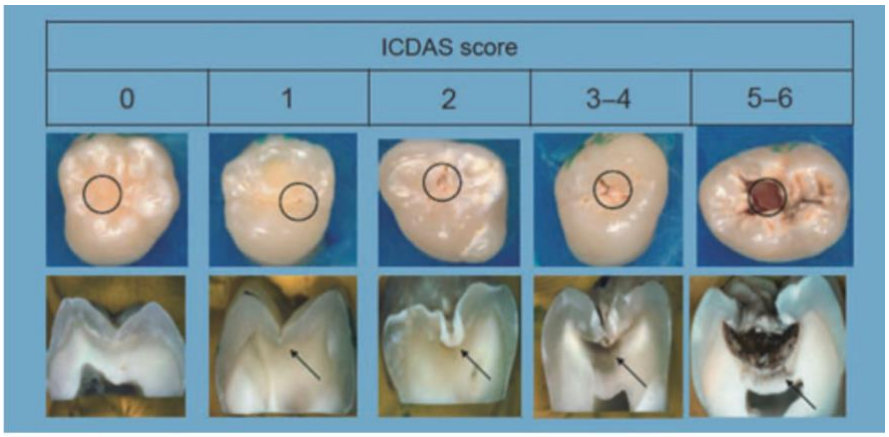

Figure 3: ICDAS codes based on the visual extension of caries lesions (16)

1.2 Periodontitis

Periodontal disease is highly prevalent, with 95% of individuals experiencing gingivitis, 60-65% having mild to moderate chronic periodontitis, and 5-15% suffering from severe periodontitis (17,18).

Periodontitis is a chronic multifactorial inflammatory disease that includes multiple causative and contributing variables (19,20). The disease involves complex dynamic interactions among specific bacterial pathogens, destructive host immune responses, and environmental factors (20).

Periodontitis, a chronic disease, is caused by the accumulation and progression of microbial biofilm at the gingival border and leads to inflammatory conditions, tissue destruction, alveolar bone resorption, and eventual tooth loss (2,12,17,21,22).

Common signs of periodontitis include gingival inflammation, clinical attachment loss, radiographic alveolar bone loss, deep probing depths, tooth mobility, bleeding after probing, and pathologic migration (20).

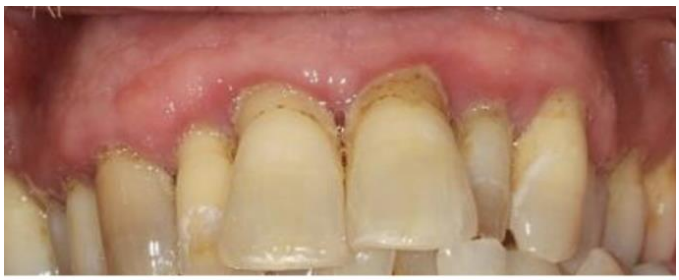

Figure 4: Picture of periodontitis with loss of attachment, gingiva inflammation and gingival recession (20).

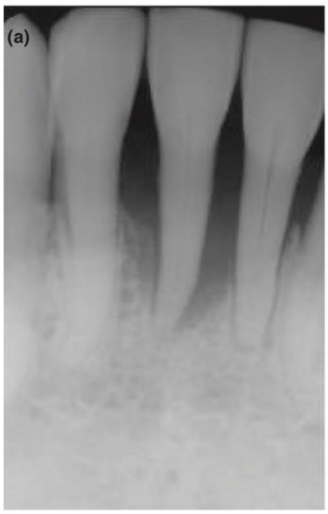

Figure 5: Peri-apical radiography of an inferior anterior teeth presenting alveolar bone loss due to periodontitis (20)

1.2.1 A multifactorial disease

For susceptible individuals, subgingival biofilm may lead to periodontal disease when pathobionts, such as specific anaerobic bacteria, develop in the biofilm (12,15,19,20,23). Periodontal disorders are complex and multifactorial conditions, which are caused by an interplay of various factors that lead to immunological

dysregulation and subsequent periodontitis (18). Factors contributing to periodontitis include subgingival biofilm, genetics/epigenetics, lifestyle-related factors, systemic disease, and other factors (17,21,23). An "ecological disaster", or growing dysbiosis, can also be brought on by a pathological and malfunctioning immunological response (21).

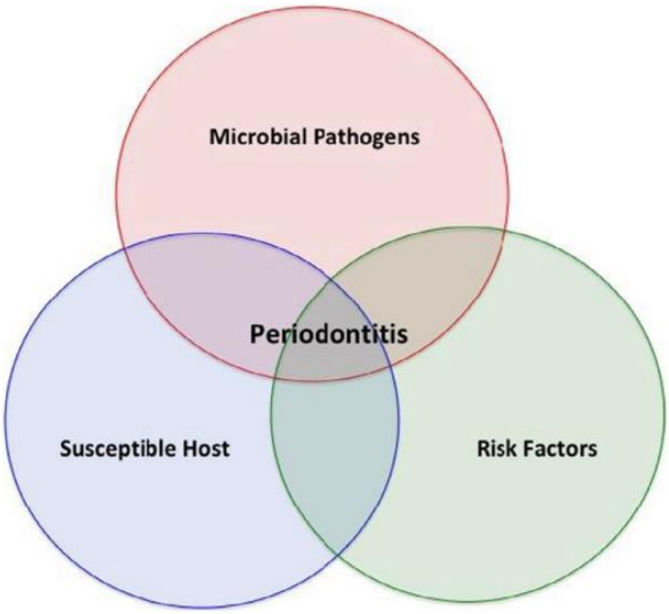

Figure 6: Periodontitis is a multifactorial disease resulting due to microbial pathogens; presence of risks factors and susceptibility of the host (20).

1.2.2 Development of periodontal diseases

Inflammation of the gingival tissue characterizes gingivitis, a more manageable and curable type of the illness (17).

Periodontitis is chronic inflammation that damages gingiva, connective tissue and alveolar bone and may arise from gingivitis in susceptible individuals (17). A change in the microbial composition and the ensuing inflammation and periodontitis lead to various potential pathogens emerging and increased host-driven tissue damage (18). Periodontitis causes the junctional epithelium to move

down, enabling the biofilm to penetrate deeper. The initiation and progression of the disease rely on alterations in the microbial community, with inflammation enriching specific pathobiont species (24). This is the vicious circle of the periodontitis (21).

Periodontitis is a chronic inflammatory disease that involves complex, interrelated events causing phases of stability and progression (21).

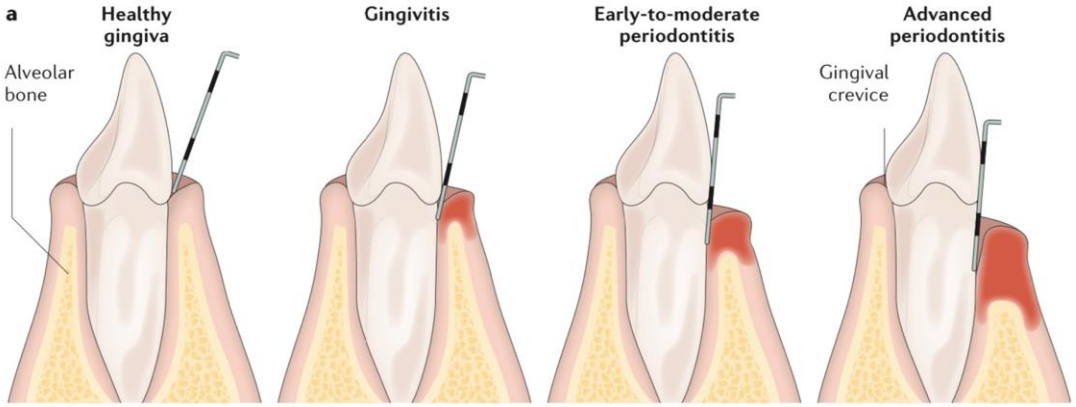

Figure 7: clinical representation of the evolution of gingivitis to periodontitis (22) .

1.2.3 Diagnostic and classification of periodontal diseases

Aggressive and chronic periodontitis were once considered separate diseases, but clinical features alone were used to diagnose them, and there is no evidence to support their distinction (19,24).

According to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, a new classification of periodontitis was established (20,24): Probing depth is not enough to diagnose periodontal disease. Other clinical indicators like bleeding on probe and factors like modifying and predisposing factors are also important (18). The etiology, disease activity, and degree of tissue damage would all be included in a classification system with a

prognosis and therapy focus (19). That's why before assessing periodontal health, gathering a patient's medical history is important to identify any systemic or environmental risk factors (20).

Three distinct types of periodontitis have been recognized based on pathophysiology (24):

- Necrotizing periodontitis: characterized by a history of pain, ulcerations, fibrin deposit (24).
- Periodontitis as a direct manifestation of systemic disease (24).
- Periodontitis is classified based on stages and grading, with different clinical manifestations and management required for each (24).

Staging and grading classification (20,24):

- Staging: it classifies the severity and extent of periodontitis and evaluates the complexity of treatment required (20,24).
    - I: It is the early stage of loss of attachment. It represents the phase between gingivitis and periodontitis (24).
    - II: It is the established periodontitis where is it possible to identify typical damages of the periodontium (24).
    - III: It shows significant damages and if left untreated, it can result in tooth loss (24).

- IV: This stage causes significant tooth loss and can lead to masticatory dysfunction (24).

Periodontal stage		Stage I	Stage II	Stage III	Stage IV
Severity	Interdental CAL at site of greatest loss	1 to 2 mm	3 to 4 mm	≥5 mm	≥5 mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (15% to 33%)	Extending to middle or apical third of the root	Extending to middle or apical third of the root
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local	Maximum probing depth ≤4 mm. Mostly horizontal bone loss	Maximum probing depth ≤5 mm. Mostly horizontal bone loss	In addition to stage II complexity: • Probing depth ≥6 mm • Vertical bone loss ≥3 mm • Furcation involvement Class II or III • Moderate ridge defect	In addition to stage III complexity: Need for complete rehabilitation due to: • Masticatory dysfunction • Secondary occlusal trauma (tooth mobility degree ≥2) • Severe ridge defect • Bite collapse, drifting, flaring • Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	Add to stage as descriptor	For each stage, describe extent as localised (<30% of teeth involved), generalised, or molar/incisor pattern			

Table 1: Representation of the four stages of periodontitis depending on the severity, the complexity, and the extension of the periodontitis (20).

- Grading: It represents the rate of progression of the disease, which is determined by the patient's history in addition to the presence of risks factors (20,24).

Periodontitis grade			Grade A: Slow rate of progression	Grade B: Moderate rate of progression	Grade C: Rapid rate of progression
Primary criteria	Direct evidence of progression	Longitudinal data (radiographic bone loss or CAL)	Evidence of no loss over 5 years	<2 mm over 5 years	≥2 mm over 5 years
	Indirect evidence of progression	% bone loss/ age	<0.25	0.25 to 1.0	≥ 1.0
		Case phenotype	Heavy biofilm deposits with low levels of destruction	Destruction commensurate with biofilm deposits	Destruction exceeds expectation given biofilm deposits; specific clinical patterns suggestive of periods of rapid progression and/or early onset disease (e.g. molar/ incisor pattern; lack of expected response to standard bacterial control therapies)
Grade modifiers	Risk factors	Smoking	Non-smoker	Smoker <10 cigarettes/day	Smoker ≥10 cigarettes/day
		Diabetes	Normoglycaemic/ no diagnosis of diabetes	HbA1c <7.0% in patients with diabetes	HbA1c ≥7.0% in patients with diabetes

Table 2: It represents the three gradings of periodontitis, depending on the rate of the progression, risks factors, the possible systemic possible impact, and the biomarkers of the disease (24).

1.3 Protection mechanisms

1.3.1 Saliva

Saliva presents many features to protect the oral tissues and maintain a healthy oral medium: its flow may encourage a mechanical removal of microorganisms from the oral cavity (1,8). On the other hand, to maintain a symbiotic environment, it may be allowing some commensal microbes to stick to the dental biofilm (1).

Saliva has antibacterial properties and functions as a part of the innate immunity in the oral cavity (4,8). Salivary components can prevent microbial colonization and control plaque biofilm population to prevent caries (1). It contains at least 45 different antimicrobial peptides and proteins (AMPs) (1,7,25).

The salivary pellicle, containing buffering compounds such as carbohydrates and phosphates, prevents demineralization of the enamel surface (1,8) and can promote remineralization of the tooth (1).

1.3.2 Microbiota

The oral cavity is one of the most intricate ecosystems and contains the most variety of biological niches in the human body (2,4). There are over 700 different species of bacteria (2,3,7) The oral microbiome has symbiotic relationships that promote a healthy balance with the host (25).

Numerous tasks carried out by the human microbiome are advantageous to the human host (2). Local bacteria serve as a biological and physical barrier to stop external organisms from colonizing or infecting the area (2). Commensal microbiota significantly influences the formation and conditioning of local immunity, and bacteria can regulate immune cell functions (4). This helps to

achieve a suitable balance between pro- and anti-inflammatory activities in the absence or during infection, which in turn helps the innate and adaptive host immune systems mature (2). The commensal microbiome has also been linked to oral mucosal wound healing (4).

1.3.3 Tissue integrity

Dental pulp, which is a loose mass of connective tissue located in the center of the tooth, is shielded by a layer of highly mineralized enamel that forms an impervious barrier against invasion by microorganisms (11,26).

Due to its integrity, the oral mucosa is impermeable to many bacteria and acts as a mechanical barrier to prevent their infiltration into tissues (4). Gingival architecture acts as an innate immunological barrier to keep out environmental infections, exogenous chemicals, and to withstand mechanical stress (1). Oral epithelial cells (OECs) that constitute the oral epithelium and its associated lamina propria act as a physical barrier to protect the body from microbes and toxins thanks to their tight connections (27).

The mucosa is continually replaced because of the epithelium and the exfoliation of the cells in its outer layer (4), preventing the aggregation of microorganisms (1,4).

1.4 Immunity in the oral cavity

The host's symbiotic interaction with its microbiota is maintained by the immune system. The host microbiota adapts and encourages the immune system to be tolerant towards the commensals and beneficial members of the microbiota, whereas the immune system influences and maintains the microenvironment of the microbiota (25).

Oral cavity immunity protects against caries and periodontitis by recognizing pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PPRs) like toll-like receptors on immune cells. Toll-like receptors trigger immune and inflammatory responses to fight infections (19).

A wide range of immune cells come together to act as the first line of defense against harmful organisms. The oral mucosa is associated with lymphoid tissues that help regulate the immune response (27).

The different cellular players of immune system in the oral cavity are (1):

- Oral epithelial cells (OECs): They will have an important role in the initiation and modulation of the immune system. They are the first cells able to detect harmful microorganisms and continually interact with the bacteria of the oral biofilm (1,28). OECs present Toll-like receptors (TLRs) and NOD-like receptors that can sense and respond to the environment by releasing immunomodulatory agents and activating other immune cells. When the host is not in danger have, OECs develop an atmosphere that is naturally tolerant and supports immunological homeostasis. When pathogens compromise the integrity of the cell barrier, epithelial cells produce signals that trigger the recruitment of T lymphocytes, dendritic cells, and macrophages (27).

- Gingival fibroblasts: During the inflammation process, gingival fibroblasts promote inflammation by secreting pro-inflammatory mediators and can also produce molecules that can lead to tissue destruction (1).

- Odontoblasts: They are the first cells in tooth tissue to respond to a microbial infection thanks to its PPRs. They have the availability to initiate and promote an immune response in the dental pulp (1).

- Regulatory T Cells: They play a major role in the immune process. They can polarize immunological responses, mostly from inflammation to tolerance. Regulatory T (Treg) cells are CD4+ T cells that are particularly important for regulating immunological responses and can provide anti-inflammatory mediators. They can also modulate the functions of dendritic cells (DCs) to maintain immune homeostasis. In case of pathogenic recognition, they can recruit new immune cells in the lymphoid tissue (27).

- T helper cells (Th): There exist different subtypes and they are implicated in the modulation of the inflammation. Th1, Th2 and Th17 are the main subsets involved in oral cavity inflammation. Th17 presence and concentration is particularly associated with the tissue destruction of the periodontium (1,4,5,17,23,25).

- Polymorphonuclear leucocytes (PMNs): Neutrophils are polymorphonuclear leukocytes that act in the oral cavity. Neutrophils travel from the circulation into tissues in less than 30 minutes in reaction to pro-inflammatory and chemotactic stimuli to eliminate invasive microorganisms. They phagocytose and kill the invading microorganisms but can also contribute to a tissue destruction process (1).

- Mononuclear leukocytes:

    - Dendritic cells: they are present in a quiescent state in the lymphoid tissues, but they are also present in the oral tissues. Myeloid and plasmacytoid dendritic cells are two different subpopulations of dendritic cells, which are specialized antigen-presenting cells (27,28). Mature dendritic cells are also strong adaptive T cell immunoinflammatory response activators and regulate the immunity through the release of cytokines (1,28).

        - Oral Langerhans cells (LCs): They have a sentinel role and are found as resident myeloid dendritic cells in the oral tissues and are constantly exposed to the oral microbiome. Under physiological circumstances, they keep the immune system in a state of tolerance. LCs start adaptive immune responses when pathogens enter the oral mucosa (27,28).

    - Resident macrophages: They are present in the oral mucosa, and during inflammation, their number considerably increases. They can mediate in a pro- or anti-inflammatory responses (1,27).

    - Monocytes: They will be attracted to the site of infection during the inflammation process and will differentiate into macrophages that may have a pro-inflammatory or a regulatory role. During chronic inflammation, monocytes may differentiate into osteoclasts that can lead to bone destruction (1).

- Natural killer cells: They interact with dendritic cells that mediate a T cell-based immune response either through direct cell-cell contact or with production of cytokines (28).

- Oral mucosal lamina propria-progenitor cells (OMLP-PCs): They primarily act in the healing of tissue, but they can also produce proteins that have an antibacterial role (27)

These cellular players are continually stimulated by different chemical mediators that play a role in the immunoinflammatory responses and inducing peripheral immune tolerance. Cytokines have a major role in the induction and control of the inflammatory response (28). They form cellular networks and interactions between immune cells such as macrophages, natural killer cells, neutrophils, T-

cells, B-cells, fibroblasts, and epithelial cells, while other interactions are regulated by cytokines (19).

The different chemical mediators that we can find during the immune response in the oral cavity are cytokines, chemokines, proteolytic enzymes, and prostaglandins (17).

Chemical mediators have different functions (17,19):

- Pro-inflammatory mediators: when released, they produce effects on various cell types of the immune system and create an inflammatory cascade (5,17). Prolonged activation or overproduction of these chemokines can lead to the activation of osteoclasts and matrix metalloproteinases (MMPs) that try to fight the progression of microorganisms along with the destruction of local tissues (5,19).

- Anti-inflammatory mediators (5) : they promote the activation of macrophages into "repair cells" and play a role in B-cell maturation and proliferation for antibody production and control of pathogenic microorganisms. An example are the interleukins 10 (IL-10) that play an essential role in the modulation of the inflammation (19).

- Transforming growth factor-beta: it controls cell adhesion, apoptosis, and wound healing as well as immunological functions and cell proliferation (19).

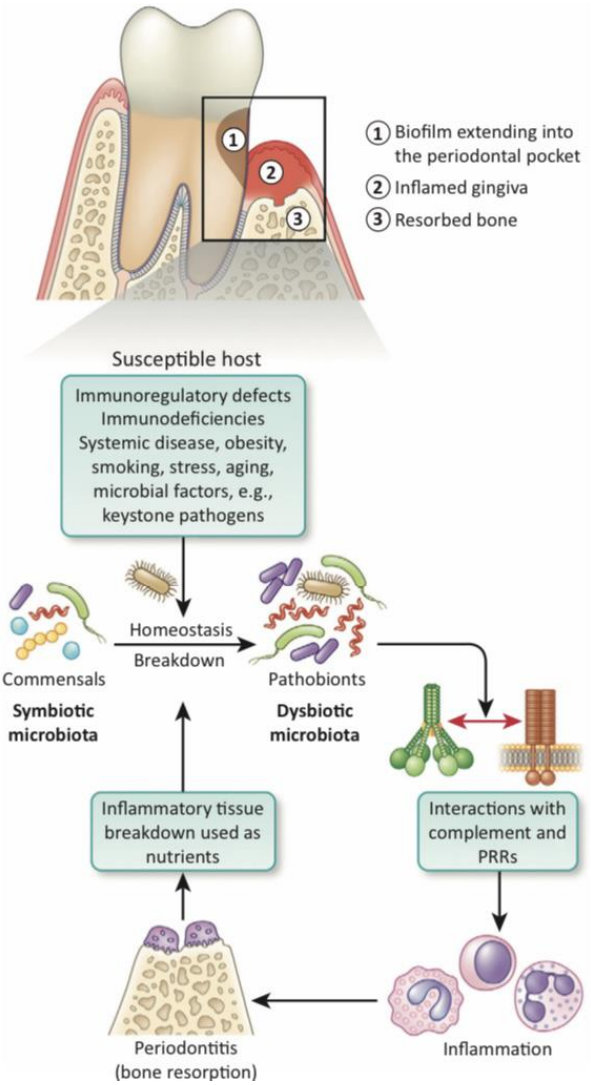

Figure 8: Recognition of pathogenic bacteria and initiation of inflammation in periodontitis (23).

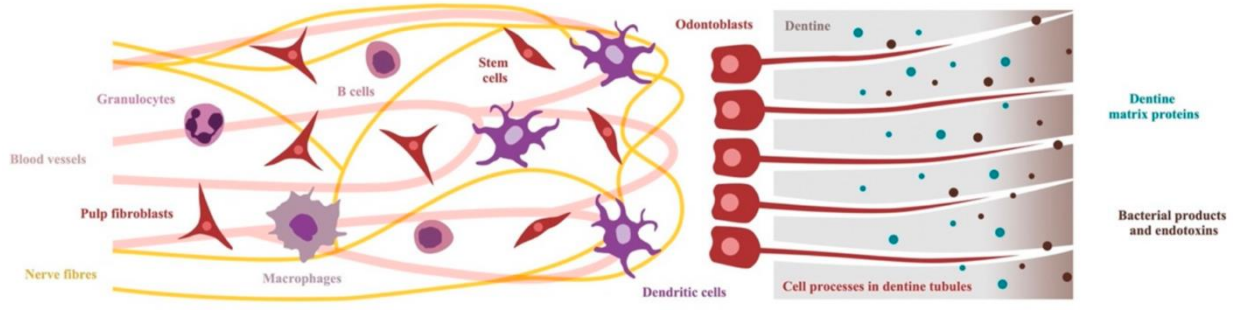

Figure 9: Recognition of pathogenic bacteria and initiation of inflammation in caries (26).

1.5 Justification

A good understanding of how defense mechanisms and immune reactions work in caries and periodontitis can help us as dental professionals to focus on the best preventive / treatment strategies and potentially found new therapeutic approaches by modulating the immune response to maintain a balanced homeostasis, providing maximum protection while avoiding the potential self-harmful effects (1,5,12,26).

1.6 State of the art:

Recent tools have allowed for a better understanding of the different etiological factors that contribute to the development of caries and periodontitis. These tools include the sequencing of the oral microbiome implicate in these diseases, as well as the involvement of genetics and systemic diseases in the dysregulation of the immune system. However, many mechanisms that mediate the immune response are not fully understood, particularly regarding the pathogenesis of the periodontitis (4,11,12,17).

2 OBJECTIVES

2.1 Main objectives:

To analyze the different processes put in place by our organism to protect us and respond to the appearance of caries and periodontitis.

To understand which cellular and chemical elements of the immunity are involved in the protection of the oral cavity.

2.2 Secondary objectives:

To evaluate whether protective mechanisms are always beneficial for the host.

To evaluate whether specific protection mechanisms exist for caries and for periodontics, respectively.

To understand if there is a synergistic protective effect between the different mechanisms in the oral cavity.

3 MATERIALS AND METHODS

3.1 Eligibility criteria

The inclusion criteria for this study allowed for all types of studies and localizations, but articles needed to be based on studies of adult population and published in English.

The exclusion criteria included the publication date of the articles, which needed to be within the last 15 years. The oldest article used was published in 2008.

3.2 Information sources

The databases used for this systematic review were PubMed (MEDLINE), the Crai Duche Chacón library of the Universidad Europea, and the tool Google Scholar. This study is based on various scientific productions, such as book extracts, articles, journals, and doctoral theses, ranging from 2008 to 2022.

3.3 Search strategy

To conduct the search for articles used for this study, keywords such as "Periodontitis"; "Caries"; "Oral immunity"; "Saliva"; "Oral microbiome"; "oral immunobiology"; and association of words using Boolean operators such as : "Caries" AND "immunity" OR "defense mechanisms"; "Periodontitis" AND "immunity "OR "defense mechanisms"; "saliva" AND "remineralization"; "Saliva" AND "immunity" OR "defense mechanisms; "oral microbiome" AND "immunity" OR "defense mechanisms"; "host response" AND "caries" OR "periodontitis"; "Microbiology" AND "caries"; "Microbiology" AND "Periodontitis"; "Cytokines" AND "caries" OR "periodontitis"; "Inflammation" AND "caries" OR "periodontitis"; "oral" AND "protecting mechanisms" were used.

4 RESULTS

The database searches yielded 29940 results and 29488 was excluded by evaluating date of publication, duplicate and eligibility criteria. After analyzing the remaining 452 articles, 14 articles were assessed for eligibility (figure 10).

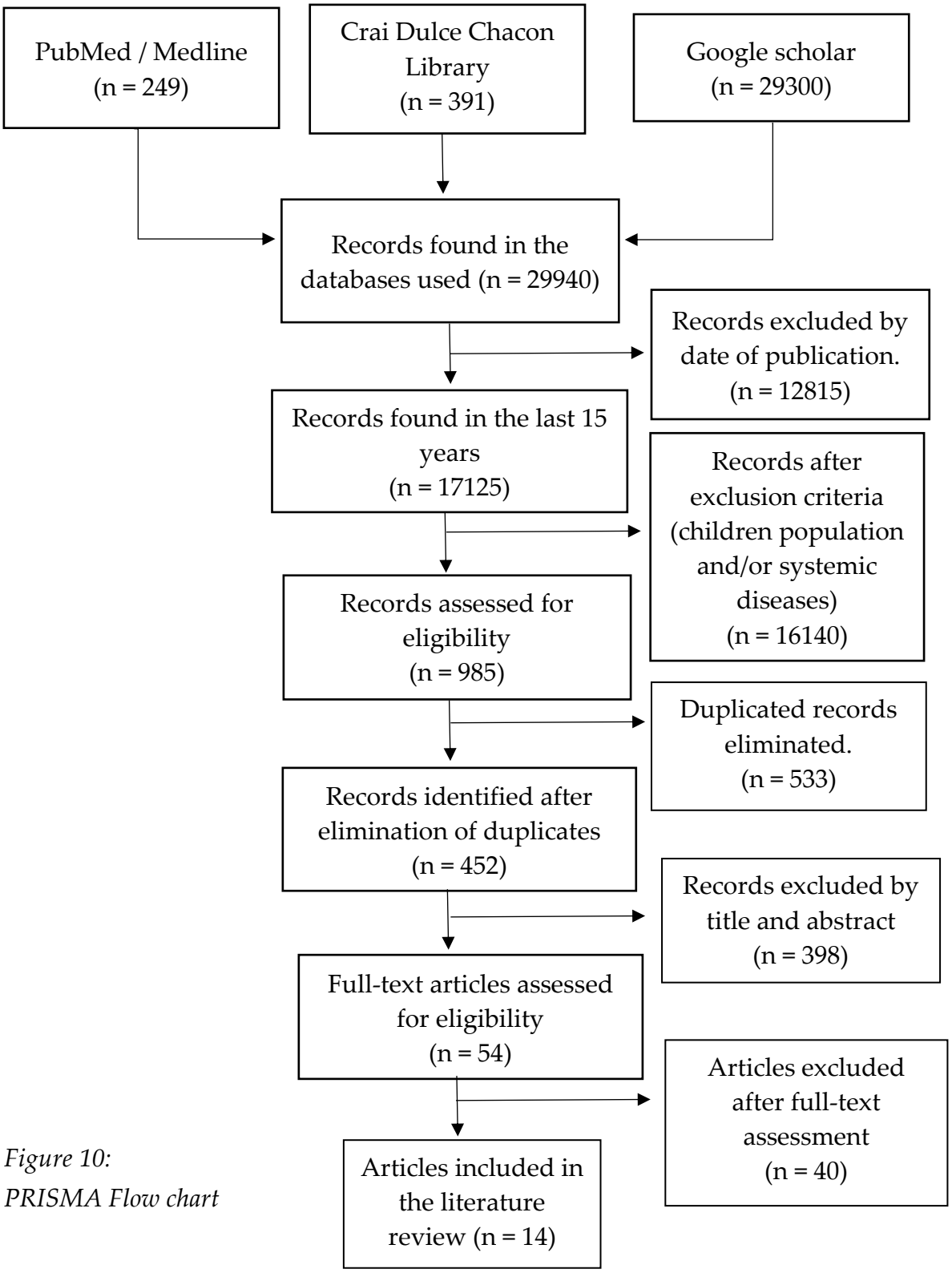

Figure 10:
PRISMA Flow chart

The 14 full texts are review studies that have been analyzed in a table format. The different sections of the table represent the information found regarding the protective function of the microbiota, saliva, oral tissues, and immunity.

The acronym "NRI" present in a column means that no relevant information was found in the article regarding the subject represented by that column (Table 4).

Table 4: Summary of information found about defense mechanisms and immunity of caries and periodontitis.

Study	Type of study	Protection functions of the microbiota	Protection functions of saliva	The different salivary components	Oral tissues as a physical barrier	Cellular immunity when detection of pathogenic microorganisms	Chemical mediators of inflammation
Meyle J, Dommisch H, Groeger S, Giacaman RA, Costalonga M, Herzberg M. 2017 (1)	*Review*	*NRI*	*-Buffering capacity & remineralization of the tooth.* *-Balance between clearance (associated to flow rate) of microbes and selective adhesion.*	*- Clearance: by mucins, agglutinin, and immunoglobulin A (IgA).* *- Homeostasis of calcium & phosphate salts by: statherin, prolin-rich, cystatin and histatin.* *- Anticaries capacity by: defensins, calprotectin, cathelicidins, histatins, peroxidase system, lysozyme, lactoferrin &*	*- Enamel with salivary pellicle: barrier against bacteria.* *- Thickness of enamel depends on the anatomic variations of tooth (thicker it is less vulnerable it is).* *- The gingival epithelium forms a barrier: tight*	*- Fibroblasts: release pro-inflammatory mediators and tissue degrading enzymes* *- Oral epithelial cells: express pro-inflammatory AMPs* *- Odontoblasts: Produce pro-inflammatory AMPs (human beta defensin 2 or hBD-2)* *- PMNs: neutrophils are attracted due to IL-1, IL-8, or bacterial peptides. They phagocytes & release ROS, tissue degrading enzymes and AMPs.*	*- Pro-inflammatory mediators: IL-1α, IL-6, IL-8, prostaglandin 2 (PGE2), IL-33, IL-34, hBD-2, calprotectin.* *- Extracellular matrix degrading enzymes: MMP-1, MMP-3* *- Regulation of tertiary dentin formation: hBD-2 and DSPP* *- Tissue destruction: dysregulation of the IL-23/IL-17 axis and/or excessive presence of reactive oxygen species (ROS).*

		secretory IgA (sIgA). *- Adherence of selective bacteria by mucin.* *- Immuno-modulatory function by: IL-1β, 17 and 23.*	*junctions of keratinocytes forms by cell adhesion molecules (CAMs).* *- Keratinized places help to resist abrasion.*	*- Hyper or hypo activated neutrophils are periodontitis characteristic.* *- Monocytes and macrophages are attracted in the infected tissue: promoting neovascularization.* *- Monocytes can mature in 2 types: M1: released pro inflammatory AMPs and tumor necrosis factor alpha (TNFα) M2: resolve inflammation. With persistent inflammation they can transforms into osteoclasts.* *- Mast cells: stimulate pro-inflammatory AMPs* *- Dendritic cells:*	*- Bone destruction: Th17/IL-17 responses (increase presence IL-17 is a marker of periodontal disease and tissue destruction)* *- anti-inflammatory mediators: IL-10*

						(Langerhans cells) modulate and/or release pro-inflammatory mediators & regulate T cells. *- Th17 cells: pro-inflammatory.*	
Valm AM. 2019 (2)	*Review*	*-Resident bacteria: chemical and physical barrier (selective exclusion).* *- Maturation of immunity (balance between pro and anti-inflammatory responses).*	*NRI*	*NRI*	*NRI*	*NRI*	*NRI*

		- Maintain vascular health of tissues with nitrate reduction.					
Garcia-Godoy F, Hicks MJ. 2008 (3)	*Review*	*-Barrier against oral noxious agents and extracellular enzymes.* *- Residents bacteria protection role: decrease effects of cariogenic bacteria (Veillonella reduce lactic acid*	*- Tooth salivary pellicle: calcium phosphate and fluoride from saliva: remineraliz ation if sufficient salivary flow* *-Fluoridated hydroxyapa tite (HAP): more acidic*	*-Statherin, prolin-rich proteins and mutinous proteins: adhesion of salivary pellicle on HAP, selective adhesion bacteria and promote supersaturation of calcium and phosphate.* *- Lysozyme: bacteriolysis.* *-Lactoferrin: inhibit growth of bacteria.*	*NRI*	*NRI*	*NRI*

		produced, Streptococcus salivarius and Streptococcus sanguinis release arginine delaminate to raise the pH.).	*resistant. -Buffering capacity. - Balance role between bacterial adhesion and bacteria clearance.*	*- Lactoperoxidase: inhibit the glucose metabolism of bacteria.*			
Ptasiewicz M, Grywalska E, Mertowska P, Korona-Glowniak I, Poniewierska-Baran A,	*Review*	*-Physiologi- cal, metabolic, and immune function for development of local immunity and keeping a balance of the immune*	*- Salivary pH and calcium phosphate concentra- tion: remineraliz ation processes of teeth. Play a role*	*- sIgA: first line of defense in the oral cavity and inhibit the bacterial adhesion to tooth and mucosal surface.*	*- Mucosal epithelium: impermeable barrier due to its structure and with exfoliation of epithelium. - The junctional epithelium*	*- Th17: hyperactivity promote tissues destruction. Recruitment of Th17 induced by IL-17 and IL-8 - Neutrophils: constantly recruited but their numbers increase during*	*- Protein GAS6: regulation of inflammation (dependent on the presence of commensal organisms).*

Niedzwiedzka-Rystwej P. 2022 (4)	*system).* *-Detoxification of toxic products (as nitric oxide).* *- Barrier against pathogenic bacteria.* *- Help in wound healing.*	*in innate immunity with anti-microbial activity.*	*(JE): highly permeable but the GCF act as a barrier.*	*inflammation.* *Different levels of activation and functions.* *- CD4+ and CD8+: important in the development of periodontal disease, producing IL-17.* *- B cells: may contribute to tissue repair or destruction.* *- Mononuclear phagocytes: may have antimicrobial functions but can also damage tissues.* *- Gingival macrophages can help in healing processes.*

Cavalla F, Araujo-Pires AC, Biguetti CC, Garlet GP. 2014 (5)	*Review*	*NRI*	*NRI*	*NRI*	*NRI*	*- Resident cells and leukocytes: TLRs for the recognition of pathogens: cells release transcriptions factors to mediate cellular response and produce inflammatory mediators.* *- Neutrophils: predominant immune cells attracted in the tissue during inflammation, they have bactericidal action with their phagocytic activity.* *- Oral epithelial cells: up regulate the production of proinflammatory mediators and*	*- TNF-α and IL-1 : pro-inflammatory. Induced neutrophils and macrophages located in the place of infection.* *- TNF-α: can induce indirect tissue destruction.* *- IL-8: chemoattraction of neutrophils. It is induced by IL-1, TNF-α and by TLRs recognition. concentration correlated with the periodontal status.* *- Chemokine ligand 2 (CCL2): regulator of monocytes mobilization &*

	leukocyte recruitment *- Gingival fibroblasts: secrete proinflammatory mediators.* *- Number of migrating macrophages is correlated with the chronicity of the response.* *- Th2: release pro and anti-inflammatory mediators* *- Th17: secrete IL-17* *- Tregs: secrete regulatory cytokines as IL-10 and transforming growth factor beta (TGF-β) (decreasing severity of the disease).*	*correlated with severity of the periodontitis* *- MMPs and receptor activator of nuclear factor kB ligand (RANKL): induced by pro-inflammatory mediators, their over-production induced tissue destruction (pathological loss attachment).* *- IL-10 & IL-4: anti-inflammatory mediator, reduces the activity of MMPs, and RANKL.* *- IFN-y: Activate macrophages (microbicidal function) and amplify*

							tissue destruction. *- IL-17 : pro-inflammatory and upregulate MMPs .*
Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, et al 2015 (11)	*Review*	*-The healthy microbiota covering the teeth is composed of non-harmless bacteria and constitute a barrier against microorganisms.*	*NRI*	*NRI*	*NRI*	*- Odontoblasts: release antimicrobial agents and proinflammatory cytokines / chemokines (BDs, nitric oxide or NO, IL-1, IL-2, IL- 8, IL-10).* *At early-stage caries: secrete a reactionary dentin. If more intense carious process they become arrested : stem cells in pulp differentiate into odontoblasts-like cells releasing deposit tertiary dentin (only when infection*	*-β-defensins (BDs) : bactericidal antimicrobial peptides, BD-2 have pro-inflammatory role (activate IL-6 and IL-8, and attract DCs, macrophages, CD4+ memory T cells and NK cells).* *- NO : potent antibacterial. Nitric oxide synthase 2 (NOS2) can promote accumulation of neutrophils, macrophages, and production of IL-8.*

	resolved). *- Immature DCs: accumulate at early stage (recruited by odontoblasts), activated during inflammation and release cytokines and chemokines (activation CD4+ T cells in Th cells or Treg)* *- Immature DCs resistant to maturation : regulatory role of inflammation, induced Treg cell and immunomodulatory mediators (like IL-10).* *- Progressive accumulation of T*	*- IL-10: attract T cells, anti-inflammatory role (decrease IL-6 and IL-8).* *- IL-6: maturation of T cells in Th2, Th17 and Tregs & increase vascular permeability.* *- Lipopolysaccharide binding protein (LBP): neutralize bacterial components, limiting the associated inflammation response.* *- IL-8: recruitment of neutrophils and pro-inflammatory mediators.* *- MMPs: degradative enzymes causing tissue damage but*

		cells, macrophages, neutrophils and B cells in pulp *- Neutrophils : attracted by IL-1, IL-2 and IL-8* *- Macrophages: phagocytes bacteria and activate T cells* *- Th1 : stimulated by IL-12 and IFN-γ, they secrete IFN-γ, IL-2 and TNF-α* *- Th2 : stimulate by IL-4 and IL-2, they secrete IL-4, 5, 6, 10, 13 and 14.* *- Th17 : pro-inflammatory role and recruit neutrophils (stimulate by TGF-β and IL-6).*	*necessary for antimicrobial action and recruitment immune cells.*

Yucel-Lindberg T, Bage T. 2013 (17)	*Review*	*NRI*	*NRI*	*NRI*	*NRI*	*-Mast cells: increase vascular permeability* *- PMNs recruited: release lysosomal enzymes.* *- In susceptible individuals: macrophages mature into osteoclasts* *- Maturation of CD4+ T cells in T helper and Tregs: Th1 release pro-inflammatory mediators, Th2 release pro an anti-inflammatory mediator, Th17 release IL-17, 23, 22, 6 and TNF-α, Tregs secrete IL-10 and TGF-β in presence TGF-β* *- Resident cells as fibroblasts & endothelial cells contribute to*	*- IL-1, IL-6, IL-12, IL-17, IL-18, IL-21, TNFα and IFN-γ are pro-inflammatory mediators.* *- IL-17: induce TNF-α, PGE2, IL-6 and IL-1 stimulating bone destruction.* *- IL-6: stimulate bone destruction.* *- IL1 and TNF: induced inflammatory mediators as IL-8, IL-6, MMPs and PGE2.* *- IL-8: characterize severity periodontitis* *- IL-4 and IL-10: anti-inflammatory mediators.* *- PGs: influence vasodilatation, vascular permeability, immunoregulatory role neutrophils and monocyte chemotaxis.*

						inflammation releasing inflammatory mediators: disease persistence.	*PGE2 (increase by IL-1 and TNF-α), they can stimulate MMPs as well as osteoclasts formation.* *- MMPs: regulate activity cytokines, contribute tissue degradation* *- Tissue inhibitor of metalloproteinases (TIMPS): control MMP activity, they have a role in tissue remodeling.* *- Monocytes: can mature into osteoclasts.*
Loos BG, Van Dyke TE. 2020 (21)	*Review*	*NRI*	*NRI*	*NRI*	*NRI*	*- Polymorphonuclear neutrophils: releasing destructive enzymes, reactive oxygen species and pro-inflammatory*	*- Treg cells: maintenance of oral tolerance.* *- MMPs: degradation collagen (released by macrophages).*

						cytokines and chemokines. When hyperactivated: stimulate bone resorption and reduced calcium uptake.	*- Pre-resolving mediators: lipoxins, resolvins, protectins, and maresins (at early stages inflammation).*
Hajisheng allis G. 2014 (23)	*Review*	*NRI*	*NRI*	*NRI*	*NRI*	*- P. Ginigivalis bacteria: modify immune response (inducing cytokines that favorite Th17 differentiation).* *- CD4+ T cells differentiation in T helper: Th1, Th2 (controlling humoral immunity,), Th17 (increase neutrophils recruitment and stimulate osteoclastic activity) and Treg*	*-RANKL: cytokine activating osteoclasts* *-IL-23: can reduce Tregs production and increase Th17.* *- IL-22: stimulated by Th17, they produce antimicrobial peptides.* *- IL-17: recruitment and activation neutrophils, expression MMPs by fibroblasts, epithelial cells and endothelial*

						(prevent excessive inflammation). *- Neutrophils: tissue destruction by releasing enzymes as MMPs, expression RANKL and releasing cytotoxic substances + induce recruitment of Th17 producing Il-17* *- B cell: released MMPs and pro-inflammatory mediators.*	*cells, and RANKL expression.*
Galler KM, Weber M, Korkmaz Y, Widbiller M, Feuerer M. 2021 (26)	*Review*	*NRI*	*NRI*	*NRI*	*- Enamel: protective barrier against microorganisms.* *- A continuous*	*- Odontoblasts : secrete antibacterial compounds, neutralize bacterial toxins, induce vascular permeability and initiate the immune response.*	*- β-defensins: antimicrobial product express by odontoblasts, epithelial and immune cells. Indirectly induced release pro-inflammatory*

	flow of dentinal fluid: avoid penetration of pathogens	*Production of pro-inflammatory mediators (IL-2, C-X-C motif chemokine ligand : CXCL1, CXCL2, CXCL8 and CXCL10), who recruit dendritic cells to neutralize bacteria toxins and inhibit dentinogenesis during severe infection.* *- Reactionary dentin is produced by odontoblast in response to a mild stimulus.* *- Reparative dentinogenesis is produced because of stronger stimuli lead to death odontoblasts*	*mediators and leukocytes recruitment.* *- Acute phase protein LBP: bactericidal and anti-inflammatory protective role.* *- cytokines: IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α control immune rep in pulp. (pro-inflammatory mediators can promote differentiation of stem cells)* *- IL-6: pro-inflammatory, induce differentiation T cells in Th17.* *- IL-10: limit inflammation intensity*

		(produce by pulp fibroblasts and resident stem cells differentiation). *- Dendritic cells: accumulate at dentin-pulp surface.* *- Pulp fibroblasts: when microorganisms pass odontoblasts barrier: pro-inflammatory mediators and produce complement effective against cariogenic bacteria (C3b protein).*	*- NO: (product by neuronal cells) induce vasodilatation, angiogenesis, tissue homeostasis and can regulate proliferation / differentiation odontoblasts. At high concentration: tissue destruction.* *- TGF-β1: stimulate reactionary dentin* *- Neuropeptides: induce vasodilatation, increase permeability and recruitment immune cells. Can also indirectly promote tertiary dentin formation by odontoblasts.*

							- TGF-β and TNF-α: can repair at low level, and destruct at higher.
Fabian TK, Hermann P, Beck A, Fejerdy P, Fabian G. 2012 (29)	*Review*	*NRI*	*-R ole in acquired pellicle formation (crystal growth homeostasis of the teeth, and physico-chemical defense of tooth). - Role in immune activation and modulation. -Anti-*	*- Salivary antibodies (sIgA): agglutination (phagocytose and cytokine production in presence of immune cells) and clearance + adhesion of specific bacteria + catalyze ozone formation (efficient microbial killing). - Salivary Chaperokine HSP70/HSPAs: cytoprotective properties, modulation of*	*NRI*	*NRI*	*NRI*

	microbial actions *- Balance between selective adhesion of bacteria and clearance by agglutinatio n.* *- Act in healing process of mucosal lesions*	*cytokine release, immunity, and modulation of neuronal function.* *- Defensins: destroy bacterial membrane, immune activator and modulator by inducing cytokines* *- Histatins: Destroy bacterial membrane and inhibit growth of bacteria + selective adhesion and stimulate healing process of mucosa.* *- Lactoferrin: Destroy bacterial membrane and inhibit growth of*

	bacteria + selective adhesion. *-Cathelicidins: destroy bacterial membrane, neutralize bacterial lipopolysaccharides (LPS) + role in healing process of mucosa.* *- Secretory Leukocyte Proteinase Inhibitor (SLPI): destroy bacterial membrane.* *- Adrenomedullin: prevent bacterial growth and destroy bacterial wall.* *-Lysozyme: destroy bacterial*

	membrane. *- BPI, BPI-like and PLUNC Proteins: bactericidal, neutralize endotoxin opsonic properties + promote clearance by agglutination and modulate the inflammatory response.* *- α-Amylase: clearance by agglutination and selective adhesion + inhibit growth of bacteria.* *- Cystatins: inhibit bacterial growth & immunomodulator y.*

	- Proline-Rich Proteins (PRPs): clearance and surface exclusion *- Salivary Mucins: selective adhesion and agglutination promoting clearance.* *- Peroxidases: lactoperoxidase and myeloperoxidase produce hypothiocyanite (bactericidal effect).* *- Statherin: inhibit adhesion of some cariogenic bacteria and promote clearance.* *- Salivary Agglutinin (SAG,*

				gp-340): aggregation for clearance and surface exclusion + antibacterial properties and immunomodulator. *- Calprotectin: inhibits microbial growth* *- LPS-binding proteins: to avoid tissue destruction by LPS.*			
Şenel S. 2021 (25)	*Review*	*-Promote oral health* *- Modulate the immune response and help it to be tolerant for the*	*NRI*	*-45 distinct AMPs are present: maintain oral health and maintain the epithelial barrier by enhancing tight junction related*	*- Orla mucosa: physical barrier consisting in stratified epithelial cells and*	*-The Th17 and IL- 17 pathway: major role in periodontitis.* *- The immune system affects and preserves healthy microbiome.*	*-Il-17: activate by T lymphocyte. They induce the release of proteins, peptides and pro-inflammatory cytokines by immune*

		commensal microbiota. *- Some bacteria: beneficial for the mucosa.*		*gene expression.* *- Defensins, cathelicidins (LL-37), calprotectins and histatins: maintain a balanced microbiota, healing wounds and chemotaxis of immune cells.*	*tight junctions that is constantly replaced. It prevents the entry of bacteria and limit its colonization.*		*cells involving the tissue remodeling.*
Costalonga M., Herzberg M. 2014 (15)	*Review*	*NRI*	*-Regulates biofilm formation and its composition*	*-Secretory IgA, mucins, agglutinin (GP340) and proline-rich proteins: selective adhesion and clearance by agglutination.* *- Cystatins, histatins, lysozyme,*	*NRI*	*- PMNs: Release antimicrobial proteins.* *- If the inflammation is not resolving the chronicity is mark by the infiltration of B and T cells.* *- In susceptible individuals: degrading*	*- MMPs : released by neutrophils and T cells, causing alveolar bone loss*

				lactoferrin, lactoperoxidase, defensins, cathelicidin, and calprotectin (S100A8/A9): provide antimicrobial function and limit the over-growth of pathogenic species.		*enzymes (proteases, elastases, collagenases and MMPs) destroy the periodontium in attempt to kill the pathogenic bacteria.* *- Th cells release pro-inflammatory mediators (leading to loss of attachment).* *- DCs and LCs: Drive the differentiation of the different subtypes of Th cells from CD4+ T cells.*	
Lang M, Zhu L, Kreth J. 2010 (12)	*Review*	*-Develop the immune defense and induce the release of anti-*	*NRI*	*-sIgA: Role in immune defense of oral mucosa.* *- Lysozyme: destroy bacterial cell wall* *- Peroxidase:*	*NRI*	*- CD4+ T cells differentiate into Th cells: influence the activation of cytotoxic T cells CTL and B cells becoming later antibody-secreting*	*-IL-1b, PGE2, IL-6 and monocyte chemotactic peptide-1: concentration corelated with severity of the disease. It induces connective*

	microbial components.	*release antimicrobial products.* *β -defensin: destroy cell membrane of bacteria.* *- Calprotectin: impede the metabolic process of bacteria.* *- Protease inhibitors: inhibits nutrients for bacteria survival.* *- Lactoferrin: inhibit bacterial growth.*	*plasma cells.* *- Macrophages: release IL-6, IL-1b, and monocyte chemotactic peptide-1 (chemoattraction of leukocytes killing pathogens by phagocytosis and other mechanisms).* *- Odontoblasts: release pro-inflammatory cytokines and chemokines like CCL20 and TNF-a with chemoattractant properties.* *- Dentinal fluid contain IgG: recognition and clearance of bacteria*	*tissues and bone loss.* *- Elastase, gelatinase, heparinase, and phospholipase: when inflammation response is prolonged and severe: release tissue damage.* *– MMPs: enzymes destroying tissue and bone.* *- CCL20: they recruit lymphocytes and dendritic cells.* *- TNF-a: stimulate phagocytosis.*

5 DISCUSSION

5.1 The oral microbiota

5.1.1 Healthy microbiota: eubiosis

The microorganisms in the oral cavity exist in a state of health known as eubiosis. However, this does not mean that there is a homogenous population of bacteria present; on the contrary, it is a highly diverse and unique ecosystem (2,4). Some of these microorganisms are normal members of the oral flora, while others are opportunistic species that can lead to systemic and oral illnesses (4). It is thought that each human carries a subset of between 50 and 200 species that usually reside in biofilms (2,12,22), and it is possible to map the different microbial niches that compose the commensal communities (see annex 1) (12,15). The diverse range of tissue surface types with different characteristics (oxygen concentration, exposure to saliva or crevicular fluid, etc.) makes this possible, and the differences in composition also reflect the defense mechanisms and immunity present in each site of the oral cavity (2,4,15).

The human microbiome carries out numerous tasks that are advantageous to the host (2). Local bacteria act as a biological and physical barrier to prevent external organisms from colonizing or infecting the area (2). Commensal microbiota significantly influences the formation and conditioning of local immunity, with bacteria having a significant impact on regulating immune cell functions (4). By achieving a suitable balance between pro- and anti-inflammatory activities in the absence or during infection, it helps the innate and adaptive host immune systems mature (2). The commensal microbiome has also been linked to oral mucosal wound healing (4).

Recent studies have discovered that some bacteria, like the genus Streptococcus dentisani, Streptococcus salivarius, and Streptococcus sanguinis, have a direct

protective role. They have the capacity to buffer plaque pH and secrete products that inhibit the growth of oral pathogens to maintain an eubiosis state (3,30).

Both the host immune system and commensal microbiota can influence one another to maintain homeostasis. Dysbiosis in the microbiota leads to a dysregulation of the local immune response at that site (25). Any changes to the oral cavity due to diet or environmental adjustments can disrupt the balance between microorganisms, leading to dysbiosis: a condition of biofilm imbalance with an overgrowth of pathogenic bacteria. (4).

5.1.2 Microbiota imbalance: dysbiosis

Dysbiosis, or the breakdown of homeostasis in the composition of microbial communities, is a typical feature of microbiome-mediated illnesses such as periodontitis and caries (2). Significant adjustments are also made within the biofilm itself (4). As dental plaque biofilms age, changing oxygen levels cause an increase in living bacteria density, leading to thicker biofilms with internal layers that lack oxygen, creating an anaerobic environment. This dynamic development leads to an increase in biofilm complexity. (2,4,15).

Regarding caries, it is a polymicrobial infection, and Streptococcus mutans bacteria species are the main bacteria implicated in the initiation of caries and a major contributor to enamel deterioration. A high level of these bacteria is present in white spots (2-4). They are the most cariogenic pathogens due to their high acidogenic properties, but they are not significantly implicated in caries progression (4). Deeper tooth decays involve acidogenic and aciduric bacteria from Streptococcus and Lactobacillus genera, working in synergy with other bacteria. The disease progression involves a diverse population of bacteria without a specific responsible species. (15).

Regarding periodontitis, a "red complex" of bacteria considered pathogens for initiation and progression are situated in the biofilm (2,21). Like caries, the development of periodontal disease is caused by a combination of different organisms situated in sub-gingival plaque (2,12). The "red complex" is composed of Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola (2,15,17,23). Porphyromonas gingivalis is considered a key factor in the disease because it can manipulate the host's immunity, and it is present in greater numbers when a patient is affected by periodontitis (12,15,23). The dysbiosis state permits the development of pathobionts, which can exacerbate the inflammatory responses in a vicious cycle, causing the condition to progress to chronicity (21)

5.2 Saliva

Saliva quality and flow rate are important factors in remineralizing early carious lesions and in the development of dental caries (1). A high flow rate can mechanically remove microorganisms from the oral cavity and support the fluid's anti-caries function (1,3). Large glycoproteins and attached microorganisms form an agglutinated mass, which is regularly ingested, reducing the number of microbes that are retained and provide physical clearance (1,29). However, to maintain a symbiotic environment, some commensal microbes may be allowed to stick to the dental biofilm with the aid of mucins, called selective adhesion (1).

The salivary pellicle prevents demineralization of the enamel surface with its buffering compounds such as carbohydrates and phosphates (1,3). It can promote remineralization of the tooth (1) by capturing calcium, phosphate, and fluoride, and diffusing it from saliva to the porous demineralized surface of the tooth (1,3). These elements are absorbed by dental biofilm, which protects the enamel surface and enables remineralization after demineralization. Daily remineralization occurs, and for it to occur optimally, the enamel surface needs to be exposed to low quantities of calcium, phosphate, and fluoride over extended periods of time.

Several proteins, including slathering and proline-rich glycoproteins, bind to hydroxyapatite (HAP), protect it, and encourage the supersaturation of calcium and phosphate ions. Fluoride accelerates the rate of HAP and fluoridated HAP generation by acting as a catalyst (1,3).

Saliva has antibacterial properties and functions as part of innate immunity in the oral cavity (4). The anti-caries effects of salivary components may help prevent microbial colonization and control the development of the plaque biofilm population on tooth surfaces. It contains at least 45 different AMPs (1):

- Immunoglobulins: The main immunoglobulin found in mucosal secretions is sIgA, which is the first line of defense in the oral cavity (4,12). For example, its production is inversely correlated with caries susceptibility (15). It controls the oral flora by inhibiting bacterial adhesion to the tooth surface and mucosa (4).

- Many components of saliva play a crucial role in maintaining the balance of microbial clearance in the oral cavity. These components include mucins, agglutinin, immunoglobulin A (IgA), bactericidal/permeability-increasing protein (BPI), palate, lung, and nasal epithelium clone (PLUNC) proteins, lactoferrin, alpha-amylase, proline-rich proteins (PRPs), statherin, and agglutinin (1, 12, 15, 25, 29).

- Other products can have a direct anti-microbial effect that leads to bactericidal or bacteriostatic activity. For example, they can kill bacteria by destroying their cell wall, inhibit their growth, or impede their metabolism by capturing or releasing certain compounds. These include defensins, histatins, calprotectin, cathelicidins, peroxidase system, lysozyme, lactoferrin, sIgA, SLPI, adrenomedullin, BPI and PLUNC proteins, α-amylase, cystatins, agglutinin, and calprotectin (1,12,15,25,29).

- Additionally, salivary proteins are crucial for the control and modulation of immune responses, as they can indirectly induce the release of chemical mediators by epithelial and immune cells. Examples of such proteins include chaperokine HSP70/HSPAs, defensins, cystatins, and agglutinin (1,25,29).

- They also play a role in the recovery of mucosal lesions, with histatins being a notable example. (25,29).

The flow rate of saliva and all its components have the ultimate goal of maintaining a symbiotic state of the oral microbiome (25).

5.3 The oral tissues as a physical barrier

5.3.1 The tooth

The dental pulp is a loose mass of connective tissue located in the center of the tooth and is protected by a layer of highly mineralized enamel, along with the salivary pellicle, which serves as a barrier against microorganisms (3,11,26). If pathogenic microorganisms or substances manage to disrupt the enamel barrier, they can be flushed away by the constant flow of dentinal fluid (12,26).

5.3.2 The oral mucosa

Due to its integrity, the oral mucosa is impermeable to most bacteria and acts as a mechanical barrier to prevent their infiltration into tissues (4). The gingival architecture acts as an innate immunological barrier to keep out environmental infections and exogenous chemicals, and to withstand mechanical stress (1). The mucosa is continually replaced because of the epithelium and the exfoliation of the cells in its outer layer, preventing microorganism aggregation (1,4,25). Keratinization aids in resistance to abrasion, and saliva physically lubricates the

surfaces and offers antibacterial protection, both contributing to the gingival epithelium's protective function (1).

Despite not being keratinized, the specialized junctional epithelium (JE) expresses cell adhesion molecules (CAMs) that mediate cell interactions and generate cell-cell adhesion, serving as a barrier against pathogens (1,25). Gingival fluid passing through the JE is composed of plasma proteins, defense cells, cytokines, and immunoglobulins, which help protect tissues from infections (4).

5.4 Immune response to caries pathogens

5.4.1 The different immune mechanisms involve counteracting the pathogenic invasion.

The pulp has several complex defense mechanisms and is well-equipped to detect and repel germs and their products (26). The function of pulp cells is more obvious in deep carious lesions where bacteria can penetrate deeper beyond the dentin into the pulp after the carious process compromises the integrity of the hard surfaces (1). As bacteria multiply, they emit metabolically active substances that cause immunological, inflammatory, and antibacterial responses primarily in the dentin and later in the pulp tissue (26).

The odontoblasts are arranged in a tightly packed layer lining the pulp (12,26). Odontoblasts are essential mediators of the inflammatory and healing processes, being the earliest cells to react to bacterial elements contaminating the dentinal tubules (1,12).

If pathogens successfully demineralize the enamel barrier and invade the dentin, odontoblasts will detect it through their TLRs (1). At this point, they will produce a series of inflammatory mediators to recruit leukocytes and modulate the

inflammation. These cytokines and chemokines include IL-1, IL-8, IL-2, and IL-10, and their release triggers a chain reaction that induces more inflammatory mediators. Odontoblasts also produce antimicrobial compounds such as BDs or NO that act directly against bacteria and bacterial toxins, while also indirectly promoting the recruitment of leukocytes such as neutrophils, macrophages, DCs cells, and more (1,4,26)

Odontoblasts also induce an increase in vascular permeability to promote the rapid recruitment of immune cells to combat pathogens and stop their progression. All of these phenomena induce the up-regulation of AMPs, creating an antimicrobial "shield" at the dentin-pulp interface (1,11,12,26).

At the dentin-pulp interface, host cells start to recognize bacterial components and produce antibacterial, immunological, and inflammatory reactions (11). Numerous immune cells are present in the pulp of healthy teeth and can react first, primarily neutrophils, followed by T lymphocytes, monocytes, dendritic cells, NK cells, and B cells (11,26). During inflammation, their numbers are increased due to the recruitment process (11).

As the dentin lesion deepens, the pulp gradually and orderly deposits stem and immune cells, such as T cells, macrophages, neutrophils, and B cells. Concurrently, the pulp increases permeability and forms a vascular network (11). The recruitment of inflammatory cells and activation of odontoblasts is intended to kill microorganisms. When the stimulus from pathogens decreases to a low level, many cells produce inflammatory modulators, such as IL-10, which play a role in controlling inflammation and limiting its excessive or unnecessary reaction. Later, the pulp can produce reparative dentin to block pathogen entry (11,26).

Another protective mechanism is the generation of the acute-phase protein LBP (lipopolysaccharide-binding protein), which neutralizes components of bacterial cell walls and can also lessen the immune response by preventing the production of proinflammatory cytokines (26).

5.4.2 The tertiary dentin formation

When the stimulus induced by pathogens is mild or stopped, the formation of a new dentin interface at the pulp level forms a mineralized barrier to separate the pulp from the site of injury and bacterial invasion, which is a crucial component of pulpal defense and wound healing (11,26).

In cases of mild tooth lesion, such as early-stage dental caries, primary odontoblasts may secrete a reactionary dentin that is tubular and continuous with the primary and secondary dentin structures (1,11,26). However, in cases of stronger aggression, primary odontoblasts may die beneath a carious lesion. If the carious infection is then stopped, the pulp fibroblasts will activate stem/progenitor cells within the pulp to differentiate into odontoblast-like cells and deposit a tertiary reparative dentin matrix. The rate and quantity of new dentin produced in this process is largely superior to the reactionary dentin (11,26).

5.5 Immune response to periodontitis pathogens

5.5.1 Recognition of pathogens and resolving of its intrusion.

Inflammation plays a critical role in both defending against pathogens and healing wounds (17). The purpose of inflammatory responses is to protect the body from harmful stimuli and restore the tissue to its pre-inflammatory state and function, which is known as homeostasis (21).

The oral cavity routinely harbors diverse microorganisms, and the epithelium secretes a small number of cytokines that attract a steady flow of immune cells to prevent bacterial infiltration (5).

When bacterial infiltration occurs, it leads to gingivitis, a reversible periodontal disease that affects the gingival tissue (17). The specific immune system's sulcus and junctional epithelial cells constitute the first line of defense against bacterial threats (1,4,15). Oral epithelial cells and fibroblasts act as immunologically active sensors of the dental plaque biofilm, just as odontoblasts do with their TLRs (1,5,12,15). Once the process of pathogen recognition begins, it activates the epithelium, which increases the proliferative rate and produces a variety of antimicrobial peptide (AMP) families that may regulate mucosal microbes. It also produces autoregulatory cytokines and facilitates appropriate interactions to recruit leukocytes by increasing vascular permeability (1,5,12,15). The main inflammatory mediators are cytokines, chemokines, and prostaglandins (17).

Monocytes, macrophages, neutrophils, and dendritic cells that are present or recruited to the site of infection kill pathogens using various methods, including the release of antimicrobial compounds and phagocytosis (1,4,12).

Tissue-resident macrophages play a crucial role in maintaining the body's mucosal resistance by serving an antibacterial purpose and aiding in wound healing and tissue repair (4). Several anti-inflammatory mediators, including TIMPS and IL-10, regulate the activity of significant tissue-destructive enzymes generated by macrophages during the inflammatory response. IL-10 decreases the activity of pro-inflammatory cytokines IL-6 and IL-8, which play an important role in potential tissue and bone destruction (1,11,17). Therefore, inhibitory mediators not only play a role in the active resolution of inflammation but also in the restoration of tissue homeostasis (21).

Although tissue damage and repair often occur concurrently, the balance can shift towards tissue destruction (17). Maintaining stable gingivitis is a protective host response against pathobionts, which helps prevent the onset of periodontitis (23). Tissue homeostasis is maintained if the host response restricts the microbes spatially and in small numbers. However, if the host response is unable to combat bacterial challenges (especially when specific pathogens with virulence factors invade tissue or interfere with the host's defense mechanisms), chronic inflammation may develop, resulting in the destruction of surrounding tissues (5).

5.5.2 The responsibility of the immune system in the development of periodontitis

As seen before, inflammation is a delicate process that needs to be balanced. Some individuals, due to various factors such as the types of bacteria present in the dysbiotic dental plaque, the quality and quantity of saliva, as well as systemic and genetic factors, may be more susceptible to an imbalance of the immunological reaction (12,17,21,23). When the host's immunological and inflammatory responses are insufficient to eliminate or clear the pathogenic microbes, it will cause and maintain the disease, leading to unresolved inflammation that develops into chronic inflammation called periodontitis (17)

Chronic inflammation results from changing the ecology of the subgingival environment, which favors the proliferation of pathobionts (21). The inflammatory process is driven by these bacteria situated in the dental plaque biofilm, which may also increase the production of pro-inflammatory cytokines. For example, P. gingivalis is known to produce pro-inflammatory cytokines (1,12).

The non-resolution to kill pathogens increases the number of degrading enzymes such as MMPs, neutral proteases, elastases, collagenases, and pro-inflammatory mediators. These are responsible for the increase in osteoclastic activity and tissue destruction in an apparent attempt to kill microorganisms and resolve the situation (1,12,15). The loss of attachment caused by the increasing osteoclastic activity and soft tissue destruction increases the depth of the pocket and the penetration of pathogenic bacteria, such as the "red complex", due to the anaerobic environment (15). This leads to a vicious circle of bacterial proliferation due to a favorable environment (1,15,21). Inflammation and dysbiosis support each other (21)

The accumulation of polymorphonuclear neutrophils plays a crucial role in the development of periodontitis (1,23). Due to their hyperfunctionality, these cells are most likely to contribute to the breakdown of periodontal tissues by releasing key chemical mediators of periodontitis, such as MMPs (23).
Neutrophils are not capable of changing their activity and functions. Monocytes, on the other hand, can mature into two subtypes: M1, which releases pro-inflammatory mediators, and M2, which initially has a modulatory role in inflammation but will differentiate into osteoclasts if inflammation becomes chronic (1,4).

Due to all these events, an unbalanced release of pro-inflammatory mediators inducing direct or indirect osteoclastic activity occurs. The main mediators implicated are TNF-α, MMPs, IL-17, IL-6, MMPs, and PGE2 (1,4,5,17).

The release of IL-6 favors the differentiation of CD4+ T cells into different subsets that play a major role in the destructive mechanisms of periodontitis. CD4+ T cells can differentiate into Th cells and Tregs cells. Th cells have an

immunomodulatory role, but Th17 is the most implicated in the pathogenesis of periodontitis by releasing IL-17, which acts as a major player in tissue destruction. The differentiation of CD4+ T cells is also stimulated by IL-17, leading to a vicious cycle of tissue and bone destruction. The differentiation into Tregs cells permits the production of an anti-inflammatory effect and reduces the destructive power of Th17. That's why the Th17/Tregs pathway plays a major role in understanding periodontitis (see annex 2) (1,4,5,17,25).

6 CONCLUSION

The oral cavity presents various mechanisms to protect against caries and periodontitis. The symbiotic oral microbiota plays an important role in protecting the teeth and mucosa by excluding pathogenic bacteria. The saliva provides different physical and chemical mechanisms to prevent the accumulation of microorganisms on the dental biofilm. The oral mucosa and enamel surface of the teeth represent a physical barrier, and the immune system is constantly present to directly act against the risk of bacterial invasion.

All these mechanisms need to work synergistically to prevent the development of caries and periodontitis. The existence of periodontitis highlights the significance of the collaborative working of all these elements. When one mechanism is imbalanced, it can rapidly lead to a vicious circle, where the initially beneficial effect of the immune response could appear to be detrimental.

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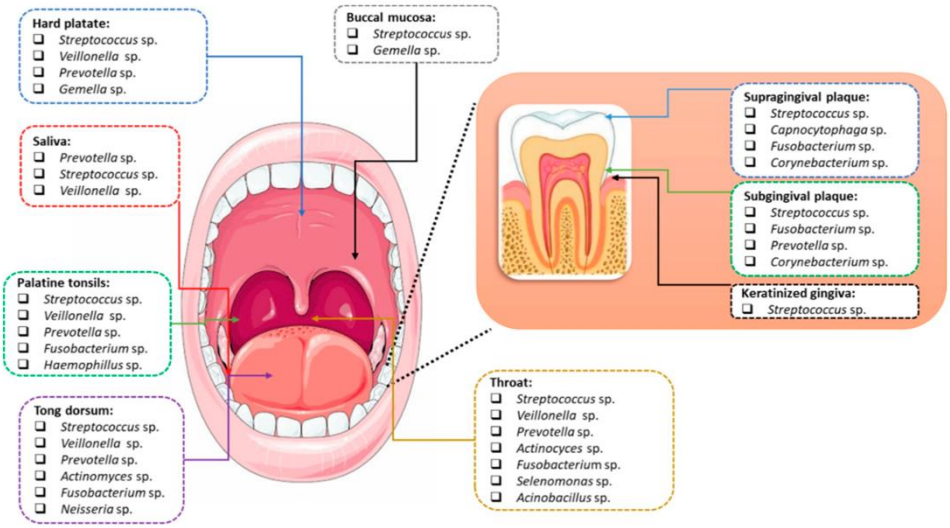
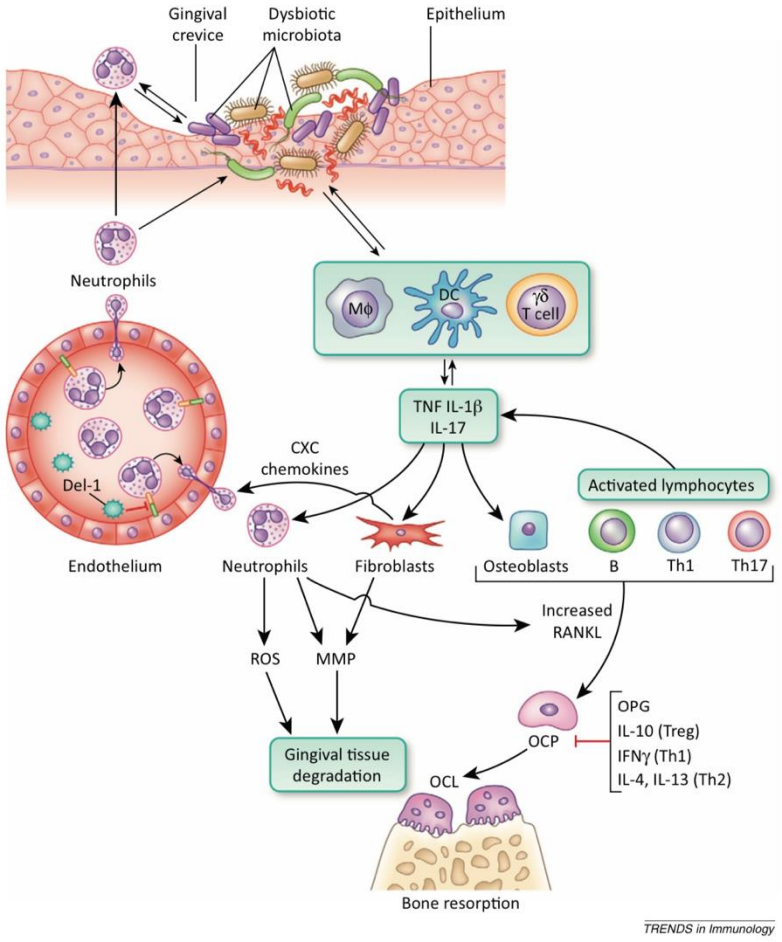
8 ANNEXES



Annex 1: Different ecological niches of the oral cavity (4)



Annex 2: Schema of periodontitis inflammation reaction (23)