Host Neuro- Immuno-Endocrine Responses In Periodontal Disease

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Abstract: Periodontitis is a chronic inflammatory complex disease caused by microorganisms. It may be influenced by diverse systemic disorders, environmental, genetic and socio-psychological factors with the ability to alter the balance of the host neuro-immuno-endocrine responses. It is characterized by the progressive destruction of the tooth supporting apparatus leading to tooth loss, with possible impact on general health.

Starting with a brief description of the periodontium, etiopathogenesis, repair processes and several physiological mechanisms and their disarray on periodontium response to bacterial challenge. Following, the negative effects of stress on the disease and some remarks on the recently discovered effects of oxytocin that modulate stress response and its role in individual coping mechanisms to stress. We also focus on the participation of components and functions of endocannabinoid system with anti-inflammatory actions on gingiva.

Finally, a discussion that may link between diabetes, cardiovascular diseases, stroke and metabolic syndrome associated with periodontal disease; all of them sharing a common denominator that is inflammation and oxidative stress.

Keywords: Gingiva, neuropeptides, inflammation, stress, endocannabinoids, cytokines.

INTRODUCTION

The existence of periodontitis goes back in time. Pathopaleodontology studies have revealed the presence of this disease in the mandible of Pre-Neanderthals found by Hans Virchov in Ehringsdorf, Germany. Tabloids with cuneiform characters found in the Library of Assurbenipal, Syria described medical treatments that Babilonians and Assyrians used to treat periodontitis. Apart from magical considerations, there were many medical suggestions for the treatment of periodontal disease using formulas containing aloe, cannabis and myrrh, for example, which were applied by fingertips to the teeth when they were loose and showed gingival swelling. In ancient Egypt, doctors specialized in different fields for treating medical issues such as those related to the head, bones, intestine, heart and teeth. Furthermore, information about periodontal disease was found in an ancient book, Susruta Samhita, describing Indian medicine from the Brahmanic period. Here a rather detailed description revealed that loose teeth were often associated with swollen and bloody gums, suppuration and an awful smell. It was suggested that these effects were due to local or systemic blood alterations [1].

In our days, world prevalence of periodontal disease is about 5% to 15%. Any population suffers from severe generalized periodontitis and the majority of adults may be affected by mild to moderate form of periodontitis [2, 3]. Epidemiological data from Argentina show that only 3% of the population is healthy and 97% need periodontal treatment: 17% oral hygiene instruction, 65% oral hygiene instruction and scaling and 14% complex treatment [4].

Although much has been learned about periodontal disease over the years, we are still trying to better understand its etiology and treatment. Periodontitis is a chronic inflammatory disease caused by microorganisms on the tooth surface and invading surrounding tissues, associated with the interactions between genetic predisposition and environmental factors that play an essential role in the development and maintenance of the pathology as in complex diseases. Complex diseases are common, chronic, associated with variations in multiple genes, each having a small overall contribution and relative risk for the disease process. These genes are considered disease modifying genes. Using RNA-seq, at least 50 up-regulated genes that are involved in the subsequent immune response were described. Therefore, genetic polymorphisms exist in many of the inflammatory and immune mediators such IL-1, IL-6, IL-8, TNF-alpha, etc. Recently, two new genes had been identified to be expressed in periodontitis: interferon regulatory factor 4 and chemokine (C-C motif) ligand 18 [5-7].

The aim of this update is to review the host neuro-immuno-endocrine responses to the bacterial challenge in chronic periodontitis and how they can be modulated by environmental and individual factors.

BRIEF REVIEW OF THE ANATOMY, FUNCTION, MOLECULAR AND BIOLOGICAL FEATURES OF THE PERIODONTIUM IN ORDER TO UNDERSTAND PERIODONTAL DISEASE

The periodontium is integrated by four tissues; the gingiva, periodontal ligament, alveolar bone and root cementum. Together they act as a unit to provide support and maintain the teeth in function. Teeth go from inside to outside the body by penetrating the integument. Gingival epithelium and connective tissue around the teeth form a dentogingival complex, which serves as unique barrier to oral challenges. The skin and mucosal surfaces are the major components of our natural immune system because they serve as physical barriers with phagocytic cells and blood-borne molecules that participate defending the organism from the external hostile environment [8].

Histologically, the gingiva is composed by epithelial and underlying connective tissue. Anatomically, it is classified in two parts: free gingiva and attached gingiva. After complete eruption of the tooth the free gingiva is the terminal edge or margin of the gingiva surrounding the teeth, usually 2mm wide and it also comprises the interdental papillae. Facing the tooth it presents a small V shaped invagination like a shallow crevice named gingival sulcus which must be maintained healthy by intense and prolonged plaque con-

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1381-6128/14 $58.00+.00

trol. In health this sulcus can measure from 1 to 4mm depth, during inflammation it becomes deeper > 4mm and it forms the "gingival pocket. The attached gingiva is continuous with the free gingiva, extends in apical direction up to the movable alveolar mucosa. It is firm, resilient and tightly bound to the underlying alveolar bone and root cementum by connective tissue fibers making it immobile in relation to the other tissues [9] (See Fig. **1a**).

The gingival epithelium shows different morphologic variations that include: the oral epithelium, sulcular epithelium and junctional epithelium. The oral gingival epithelium covers the crest and outer surface of the free gingiva, it is keratinized composed by four layers: *stratum basale* (basal layer), *stratum spinosum* (prickle cell layer), *stratum granulosum* (granular cell layer) and the *stratum corneum* (keratinized cell layer). In the oral epithelium 90% of the cells are keratinocytes. Langerhans cells, melanocytes and Merkel cells are non-keratin producing cells in the basal layer. The Langerhans cells that are dendritic cells, belong to the mononuclear phagocyte system, and play an important role in protective immunity by processing exogenous antigens and presenting them to antigen specific T lymphocytes that become activated during inflammation. Melanocytes are found interspersed between keratinocyts in the basal and spinosum stratum they synthesize melanin and the melanin granules are phagocytosed and contained within other cells of the epithelium and connective tissue called melanophages. Therefore, melanocytes are responsible for brownish pigmentation of the gingival. Merkel cells are located in the deep layers of the epithelium at the tips of the rete ridges connected to the adjacent cells by desmosomes, harbor intraepithelial nerve endings, forming the epidermal Merkel cell-neurite complexes involved in mechanoperception [9].

The basal lamina is permeable to fluids, acts as a barrier to particulate matter and has a wavy shape. Extensions of connective tissue project into the epithelium called connective tissue papillae and are separated from each other by epithelial ridges called also "rete pegs". Thus, it is a morphologic characteristic of oral and sulcular epithelium while they are absent at the junctional epithelium. During inflammation the epithelial ridges become elongated [10].

The sulcular epithelium lines the gingival sulcus, extends from the crest of the free gingival margin apically to the coronal limit of the junctional epithelium. It is a non-keratinized stratified squamous epithelium, lacking of granulosa and cornea strata. The sulcular epithelium has the potential to keratinize if the bacterial flora is totally eliminated from the sulcus. These findings suggest that the local irritation prevents sulcular keratinization. The sulcular epithelium acts as a semi-permeable membrane through which tissue fluid may pass into the sulcus and bacterial products may pass to the gingival tissues [11].

The gingival epithelial cells react to external stimuli by synthesizing cytokines, prostaglandins, adhesion molecules, growth factors and enzymes. They may produce peptides such as defensins and also eicosanoids such as endocannabinoids. During challenge, these cells will over-express adhesion molecules such as intercellular adhesion molecule-1 and cytokines, chemokines, prostaglandins, Toll-like receptors and proteolytic enzymes for polymorphonuclear cells recruitment and migration. All together, these molecules act as initiator, regulator and mediator of host immune response. Histopathologic observations demonstrating disruption of the integrity of the periodontal pocket, a sizeable ulcerated surface amounting to up to 8 to 20 cm^2 [12].

The junctional epithelium is a non-keratinized epithelium attached to the tooth surface by basal lamina and hemidesmosomes. It is located at the cement-enamel junction and it starts with one or two cells extended coronally, increasing up to 20 cells wide when it reaches the sulcular epithelium. The rapid turnover of these cells contributes to the host-parasite equilibrium and rapid repair of tissue damage. Together with the sulcular epithelium they have the hability to replenish themselves in 1-6 days turnover time and at the

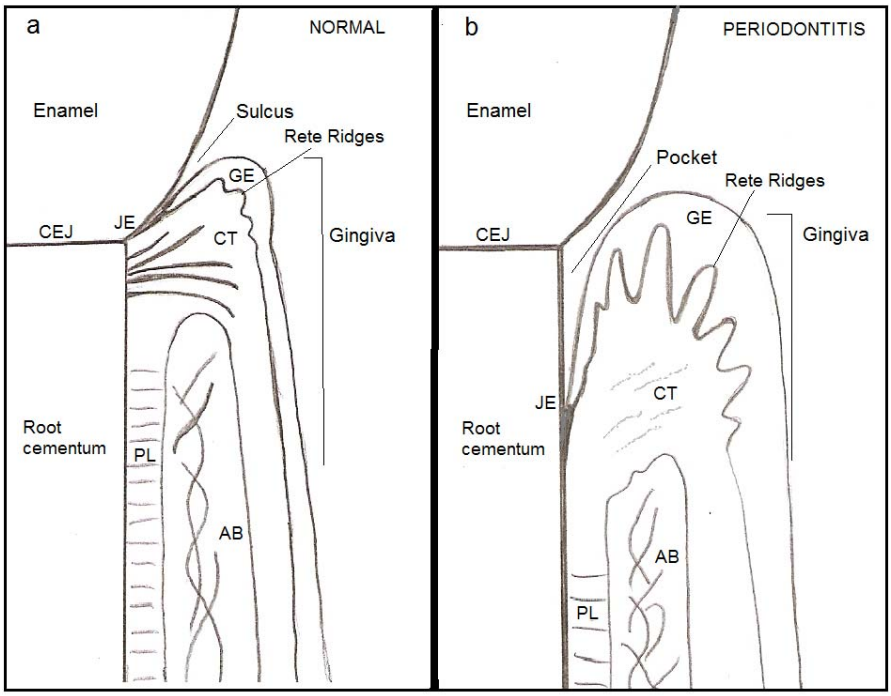

Fig. (1). Anatomical landmarks of periodontium in normal (**a**) and periodontitis (**b**).

Cemento enamel junction (CEJ), Junctional epithelium (JE), Gingival epithelium (GE), Connective tissue (CT), Periodontal ligament (PL), Alveolar bone (AB).

same time fibroblasts can also produce new collagen fibers. The regenerating epithelial cells move towards the gingival sulcus where they are shed. Gaps between the cells appear to be larger than in the sulcular epithelium. The junctional epithelium is a highly permeable, genetically programmed participating in homeostasis. Leukocytes and neutrophils predominate in the gingival sulcus and appear to migrate continuously through the junctional epithelium into the sulcus. This infiltrate increases with the accumulation of plaque and gingival inflammation. The recruitment of leukocytes from the gingival tissue to the crevice is due to the chemo attractant action of products derived from the biofilm as well as from factors released by the host. Transudates and exudates of fluid that contain varying amounts of plasma proteins leave the vessels of the dentogingival plexus and arrive in the gingival crevice region as a gingival crevicular fluid. The main rout of the gingival fluid diffusion is through the basal lamina and the wide intercellular spaces of the junctional epithelium and sulcular epithelium into the sulcus. Other components are connective tissue elements, epithelial cells, inflammatory cells, serum, and microbial flora inhabiting the gingival sulcus and margin. In health, the amount of gingival fluid is very small. During inflammation the flow increases from 20 µl per hour up to >20 µl per hour and its composition resembles that of an inflammatory exudate. It contains a vast array of biochemical factors which can be used for diagnostic or prognostic biomarkers of the biological state of the periodontium in health and diseases [9, 13].

The attachement of the junctional epithelium around the tooth surface is reinforced by the gingival fibers which brace the gingival margin. For these reasons it is considered a dentogingival unit. Below the cementoenamel junction the gingival connective tissue via collagen fibers anchor into the root cementum surface just below the terminal point of the junctional epithelium, form the connective tissue attachment. The stability of this attachment is a key factor in limiting the migration of junctional epithelium. The gingival connective tissue known as lamina propia, fixes the teeth within the alveolar bone and gives support to the epithelial tissues. The main component are the collagen fibers (60%), fibroblast cells (5%), nerve fibers and vessels (35%) and some other cells, all embedded in an amorphous substance known as matrix. Collagen fibers constitute the most essential component of the periodontium. They are organized in two patterns: one in dense thick bundles of fibers with Type I collagen and the other Type III collagen in a loose fiber arrangement which are short and thin and organized in a reticular network. Fibronectin and osteonectin are observed around collagen fibers and elastin fibers are found more in the mucosal area. The function of these gingival fibers as we mentioned above is to brace the free margin firmly around the tooth, providing rigidity to withstand the forces of mastication without being deflected away from the tooth surface [9].

There is a wide range differences in the population of the fibroblasts cells with variable features including morphology, migratory behavior and matrix synthesis. The gingival fibroblasts have mesenchimal origin, they synthesize collagen, elastic fibers, glycoproteins and glycosaminoglycans for the matrix. They participate in different regulatory processes necessary for tissue homeostasis via phagocytosis and degradation by secretion of collagenases and metalloproteinases. Hence, during tissue turnover and remodeling they allow for changes in shape and structure of the connective tissue without impairing function. Fibroblasts respond to changes in the matrix to metabolic products, growth factors and cytokines in a paracrine or autocrine manner. If the regulatory processes are altered a net gain or loss of connective tissue will result in overgrowth or tissue destruction. Transforming growth factor-beta secreted by platelets and macrophages influence the synthesis of collagen and other matrix components by fibroblasts. On the other hand, collagen synthesis may be inhibited by TNF alpha, prostaglandin E_2 and interferon-gamma at a transcriptional level. Other cells found in the lamina propia are macrophages derived from circulating blood monocytes which migrate into tissue, being numerous during inflammation. Adipocytes and eosinophils are less in number and B lymphocytes appear in greater proportions to elaborate specific antibodies against already-recognized antigens that are always present in the sulcus of clinically normal gingival [14].

The connective tissue cells are embedded in the matrix. It is the medium essential for the maintenance of the normal function of the connective tissue. Proteoglycans are the main constituent, classified in groups depending upon the location. They are found as matrix organizers and tissue space fillers and are intracellular or cells surface components. Integrins are cell surface molecules that regulate cell adhesion and migration for the epithelial cells, fibroblasts, leukocytes, macrophages and T-cells. These molecules have been shown to be over expressed during wound healing [15].

The nerve fibers of the dentogingival region are sensitive fibers from the V craneal pair, the trigeminal nerve, specifically from the superior maxilla and inferior mandibular branch. The gingival innervations are derived from fibers arising from nerves in periodontal ligament and from labial, buccal and palatal nerves. Like other tissues, the periodontium has nociceptors and mechanoreceptors which record pain, touch and pressure. Their trophic center is in the semilunar ganglion and are brought to the periodontium via the trigeminal nerve and its branches. These nerve fibers can either be myelinated and unmyelinated and are extensively distributed throughout the gingival tissue. The myelinated fibers are closely associated with the blood vessels. The unmyelinated fibers originating in the connective tissue and penetrating into the junctional epithelium contain neuropeptides at their endings. The function of these nerve fibers is not only sensory, as they also participate in the neurogenic/inflammatory alterations responding to different stimuli (chemical, mechanical and/or sensorial) by releasing peptides such as substance P, neuropeptide Y and calcitonin gene-related peptide (CGRP). Other nerve structures that can be found are Meissner-type tactile capsules and Krause-type end bulbs, which are temperature sensitives [15, 16].

The gingiva has one of the largest end blood supplies of the body. It forms distinct networks, one bounded to the oral and sulcular gingival epithelium extend into the papillary connective tissue between the rete pegs in the form of terminal hairpins loops. The other, subjacent to the junctional epithelium termed the gingival plexus. Three sources of blood supply to the gingiva can be mentioned: supraperiosteal arterioles, vessels of the periodontal ligament and arterioles which emerge from the crest of the interdental septa. Studies of gingival vasculature have demonstrated that in the absence of inflammation, the vascular network is arranged in a regular, repetitive, and layered pattern. During inflammation, the vasculature exhibits an irregular vascular plexus pattern, with microvessels exhibiting a looped, dilated, and convoluted appearance [17].

The periodontal ligament is a soft, vascular and cellular rich connective tissue which surrounds the root of the teeth and joints the root cementum to the socket wall of the alveolar bone. In the coronal direction the periodontal ligament is continuous with the connective tissue of the gingiva and is demarcated from the gingiva by the collagen bundles which connect the alveolar bone crest with the root known as the alveolar crest fibers. The cells found in the periodontal ligament are: fibroblasts, cementoblasts, osteoblasts, osteoclasts, epithelial cells and nerve fibers. In the hope to understand more about immunomodulatory properties of this tissue during inflammation, many researchers isolate and culture periodontal ligament cells. It has been suggested that periodontal ligament stem cells (PDLSC) are located around blood vessels. Recently, cultured PDLSC were found to express markers for mesenchymal cells, stem cells and pericytes. These findings are very relevant since they show that PDLSC could differentiate into osteoblasts, adipocytes and chondrocytes and have the potential for regeneration of perio-

dontal tissue. Furthermore, as pericytes, they can form a capillary like structure and interact with endothelial cells. Another recent study shows that during inflammation, mesenchymal stem cells derived from PDLSC presented dysfunctional immunomodulatory properties. They found that during inflammation PDLSC had significantly diminished inhibition of T-cell proliferation. Furthermore, suppression of Th-1 differentiation and IL-17 production by inflamed PDLSC was significantly lesser than by healthy cells. This may contribute to osteoclastogenesis and alveolar bone loss in periodontitis [18 - 20].

Chronic periodontitis is prevalent in adult patients but can occur in children. Although, there is no clinical evidence that periodontal disease in elderly subjects is caused by the aging process, some experimental evidences suggest that aging decreases the ability of periodontal ligament cells to proliferate and modulate the expression of important inflammatory and an anti-resorptive profile. Age could be viewed as an increased opportunity for exposure for diseases and for heavy smoking. Aging might affect other aspects of managing periodontal health like reflect a cumulative exposure to a number of potentially destructive processes such as plaque associated periodontitis, compliance with supportive maintenance treatment. Interestingly, a study mentioned a greater compliance in older patients than in younger. Other authors point out that with age there are changes in immune system that show an increase in oxidant and inflammatory compounds [21, 22].

The host response is protective through surveillance, interception and removal of foreign materials and there must be a balance between repair and destruction to maintain an adequate homeostasis and normal function. If the response is not enough or inadequate, it becomes destructive rather than protective, the tissue function is compromised and clinical features as "pocketing" are observed. Periodontal pockets are not evident at simple visual inspection. This condition occurs slowly, mostly is not painful, asymptomatic, and many patients are unaware until the condition has progressed enough to express the characteristics of advanced periodontitis such as: gingival erythema and edema, gingival bleeding, gingival recession, tooth mobility and/or drifting, suppuration from periodontal pockets and at the end, tooth loss. All can occur in a variable time, during the years of chonicity and alterating with acute stages depending on the interaction of the environmental factors on individual genetic phenotype and bacterial heterogenecity [23] (See Fig. **1b**).

The progressive lesions of gingival/periodontal tissues are divided in four stages: initial, early, established and advanced. The initial and the early stages characterize the histopathology of a clinically healthy gingiva to gingivitis which can be reversed with good oral hygiene and/or periodontal treatment. The established lesions feature chronic gingivitis. The advanced lesion is when gingivitis progressed to periodontitis and the lesion is consistently associated with the attachment and alveolar bone loss. Briefly, as biofilm continues apical down growth, in $1mm^3$ of dental plac there can be 10^8 bacterias, pocket deepens and an anaerobic ecological niche is established at 4mm or more. The damage to the collagen fibers is extensive. The pocket epithelium migrates apically from the cementoenamel junction and there are widespread manifestations of inflammatory and immunopathological tissue damage. The lesion is not only localized at the gingival tissues, since the inflammatory cell infiltrate extends laterally and apically into the connective tissue of the attachment apparatus. At this stage, professional treatment with root planning, treatment with antibaiotics, surgery and good oral hygiene and maintenance are required for the treatment of this chronic disease [15, 23].

The repair involves similarity to any other tissues. These processes consist of the removal of degenerated tissue debris and the replacement of tissue destroyed by disease. The healing lesions imply regeneration and repair of the periodontal structures. It involves the remodeling of the attachment apparatus but not the regaining of the attachment level or new bone height. In periodontium one of the wound edges is the root surface, so upon injury as in a periodontal flap or after periodontal root scaling treatment, undamaged epithelial cells from the *stratum basale* start within hours to migrate to the wound margin. This migration is provided by locally released factors such as epidermal growth factor, platelet-derived growth factors, fibronectin and cytokines. This epithelialization leads to the migration of the epithelium along the root and over the wound surface apically until it reaches the collagen attached fibers on the root. This wound healing explains the formation of epithelial adaptation. The process involves inflammatory and fibroblast cells and a new synthesized matrix. Within hours, in the wound a fibrin clot adheres to the root surface with a big infiltration of neutrophils. At day 3, tissue is still well infiltrated with inflammatory cells and some fibroblast can be seen at the granulation phase starting with the fibrin clot degradation. By day 7, the granulation tissue is well formed with collagen fibers aligned in a parallel array along the root surface and the matrix continues to remodel. At day 14, some fibers may show some signs of attachment under the epithelial junction. A minimum of 3 weeks are needed for healing and fully functional connective tissue attachment to the root surface. The above mentioned epithelial adaptation allows the reestablishment of a normal gingival sulcus at the same level of the pre-existing pocket base [9].

THE ETIOLOGY OF PERIODONTAL DISEASE

The pathogenesis of periodontal disease is initiated by bacteria that colonize the tooth surface, gingival sulcus and the inflammatory-immune response of the host. Skin and all the interface surfaces of our body are exposed to colonization of many different microorganisms. Renewal by shedding of the surfaces prevents the accumulation of large masses of these organisms. Tooth surface is a hard non-shedding surface where bacteria may develop and deposit in large amounts. Thus, any deposit in the oral cavities, on any hard surfaces are named dental plaque or bacterial plaque and is considered the primary cause of gingivitis, periodontitis, and periimplantitis. Following immersion of hard, non-shedding surface into the fluid environment of the oral cavity, adsorption of macromolecules will lead to the formation of a biofilm. Therefore, dental plaque is a natural occurring microbial deposit that represents a true biofilm which consists of bacteria in a matrix composed mainly of extracellular bacterial polymers and saliva and/or gingival exudate products. It is estimated that about 700 different species are capable to colonize the mouth and any individual may harbor 150 species or more. Counts in subgingival sites range from 10^3 in healthy shallow sulci to $>10^8$ in deep periodontal pockets. In a patient with a moderate to severe periodontitis the affected area may reach up to 50-70 cm^2 [24].

The inflammatory reaction, in response to plaque bacteria is characterized by an initial increase in blood flow, enhanced vascular permeability and the influx of cells from the peripheral blood to the gingival crevice. The polymorphonuclear leukocytes (PMN) migrate through the epithelial lining to the gingival pocket to be the first line of defense against invading bacteria. These cells are nonspecific phagocytes and constitute a nonspecific acute and rapid defense. The host defense against periodontopathic bacteria comprises innate and acquired immunity. The innate immune system is activated immediately and is responsible for the defense for few days before acquired immunity with adequate cellular and humoral response occurs. The microbial pathogens present pattern recognition receptors, known as pathogen-associated molecular patterns (PAMPs), and are shared by pathogens but not expressed by the host. The host expresses Toll-like receptors (TLR) that recognize PAMPs. There are at least 10 toll-like receptors (TLR) that are the major class of signaling receptors recognizing conserved bacterial and viral structures. All TLR contain a common extracellular leucine-rich domain and a conserved intracellular domain. Activation of TLR results in a signal that is transmitted through a chain of

signaling molecules that activate the nuclear factor kappa B (NF-kB) and activated protein-1 (AP-1) that leads to transcription of genes involved in the synthesis and release of pro-inflammatory cytokines. In periodontal tissue, gingival fibroblasts and periodontal ligament fibroblasts constitutively express TLR-2 and TLR-4,and produce various inflammatory cytokines such as IL-1, IL-6 and IL-8 when stimulated by bacterial lipopolisaccharide (LPS) such as those from *Porphyromonas gingivalis* (P.g), *Tannerella forsythia* (T.f), *Aggregatibacter actinomycetemcomitans* (A.a) and *Prevotella intermedia* (P.i) [25, 26].

In general, TLRs are expressed by monocytic cells as well as by endothelial, epithelial and various other cells including gingival fibroblasts. The specificity of TLRs includes recognition of peptidoglycan (PGN), lipoproteins, LPS, atypical LPS, fimbriae by TLR-2, CpG motifs of bacterial DNA by TLR-9, as well as heat shock proteins and LPS by TLR-4. During the onset of periodontal disease (PD), the first cell type exposed to be stimulated by PAMPs is the epithelial cell. These cells express TLR-2, TLR-8 and TLR-9 and when stimulated express intercellular adhesion molecule-1 (ICAM-1), lymphocyte function-associated antigen-1 (LEA-1). Other host cells are also activated, such as endothelial, macrophages, neutrophils that produce matrix metalloproteinases (MMPs) and Langerhan cells, that acts as "centinels of innate system" looking for antigens. They can present antigens in a major histocompatibility complex and cytokines and also co-stimulatory molecules such as CD40 and CD54 that induce activation of T-lymphocytes, resulting in Th-1 or Th-2 responses. As inflammation progresses and gains access to connective tissue, macrophages are stimulated and produce many cytokines, nitric oxide, MMP-1 etc. Also, endothelial cells when stimulated can produce cytokines and adhesion molecules [27].

There are two kinds of fibroblasts in periodontium. Gingival fibroblasts and periodontal ligament (PDL) fibroblast have distinct phenotypes. Gingival fibroblasts can produce many cytokines and adhesion molecules in response to different PAMPs. PDL fibroblasts present alkaline phosphatase activity similar to osteoblasts. These cells also can secret proteinases that produce degradation of soft and mineralized tissues. There are also cementoblasts in PDL that when stimulated by LPS exhibit a decreased level of the receptor activator of NF-kB ligand (RANKL) and increased expression of the soluble decoy receptor osteoprotegin (OPG). The osteoblasts when stimulated by LPS can produce pro-inflammatory cytokines and biological mediators involved in bone resorption [28].

In gingival tissue there are also non-resident cells such as neutrophils, monocytes, B and T lymphocytes. They all can produce cytokines, chemokines, adhesion molecules etc. All these substances form a very complicated network that can shift the Th-1 to a Th-2 response. Therefore, the response is not only dependent on the composition of microbiota and the virulence of the microorganism but also on the host response that is influenced by genetic and environmental factors [29].

The recognition that periodontitis involves an inflammatory component as well as altered bone metabolism has given a new perspective on the etiology, that integrates the disciplines of immunology and bone biology and has given rise to a new discipline "osteoimmunology". During inflammation, LPS specifically increases osteoblastic expression of the RANKL that is also expressed by other cells, such as fibroblasts, T and B lymphocytes, dendritic cells or monocytes. RANKL could bind to its receptor RANK which is expressed on osteoclast precursor cells and also could bind to OPG. It is considered that bone resorption and formation are regulated by the RANKL/RANK/OPG axis. In physiological conditions there is a balance between bone resorption and bone formation. A relative decrease in RANKL concentration and increase in OPG will result in excessive formation of bone. On the other hand, a relative increase in RANKL and decrease in OPG which reflects the RANKL/OPG increased ratio and will result in bone resorption. In a recent revision of the RANKL/OPG system in clinical periodontology the authors concluded that an increased RANKL/OPG ratio may serve as a biomarker that denotes the occurrence of periodontitis, but may not necessarily predict on-going disease activity [30].

STRESS AND PERIDONTITIS

The stress concept was first described by a Hungarian physician named Hans Selye (1907-1982). As a medical student at Charles University in Prague, he observed that patients suffering from different diseases often exhibited identical signs and symptoms. Later, he described the general adaptation syndrome referred to as stress. Life exists by maintaining a complex dynamic equilibrium, or homeostasis, that is constantly being challenged by intrinsic and extrinsic stressors. Therefore, stress can be defined as a state of threatened homeostasis, which is re-established by complex physiological and behavioral adaptive responses of the organism. If the adaptive response is inadequate or excessive such that homeostasis is not re-established, then pathological situations can develop [31-33].

Gingival tissue surrounding teeth acts as a natural barrier protecting the interior of the body. Tissue damage during periodontal disease is associated with localized inflammatory response. It is well known that several chemical substances are released following tissue damage and they are able to modulate excitability of nociceptors, which can make them more sensitive to stimuli. For example, tissue damage releases prostaglandins, serotonin and bradykinin which activate and sensitize nociceptors, inducing a noxious signal that, in the region of the head, travels afferently in trigeminal nerve branches toward the trigeminal ganglion. Importantly, substance P is a 11 amino acid neuropeptide residue discovered by Ulf von Euler in 1931. It is produced by brain and peripheral neurons located in the trigeminal ganglion (TG) and it is released by the nerve terminal in an antidromical way. Thus, activation of one branch of the nociceptor fiber leads to the activation and release of substance P by other branches in the area.It is noteworthy that substance P is observed in nerve fibers of dental pulp and periodontal tissues of several species, including humans. These nerves are sensitive nociceptive small diameter unmyelinated C-type fibers. The biological effects of substance P are induced following binding to G protein-coupled receptors, the principal one for this peptide being the neurokinin-1receptor (NK-1r) a receptor known to be present in high quantities in dental tissue. Once released substance P can bind to NK-1r in mast cells in the vicinity of the sensory nerve endings; hence, stimulating the release of histamine and therefore, further exciting the nociceptors. Besides mast cells, other cell types in the region such as lymphocytes and macrophages express NK-1r and thus, can be stimulated by substance P to produce and secrete inflammatory mediators like prostaglandins and pro-inflammatory cytokines such as interleukins 1 and6, as well as TNF-alpha. In addition, substance P causes dilation of the blood vessels in the area and the edema produced further induces bradykinin release. Taken together, the resulting secretion of these substances continues to stimulate peripheral substance P release and therefore it could be one of the actors responsible for the maintenance of the local inflammatory response and chronicity of the disease [34, 35].

In addition to the above described peripheral responses to noxious signals and the release of substance P derived from neurons within the peripheral branch of TG, the central branch of the trigeminal nerve leaving the TG relays some of these inputs into the brain stem, at the level of the pons. Figure **2** summarizes peripheral/periodontal signaling, as well as the pain pathway to the brain. After entering the pons, the fibers synapse and ascend cranially in the trigemino-thalamic nerve tract. This sensory information eventually reaches many brain regions, including the amygdala and hypothalamus. With regard to the amygdala, sensory information enters the basolateral nucleus where it is integrated, then relayed to

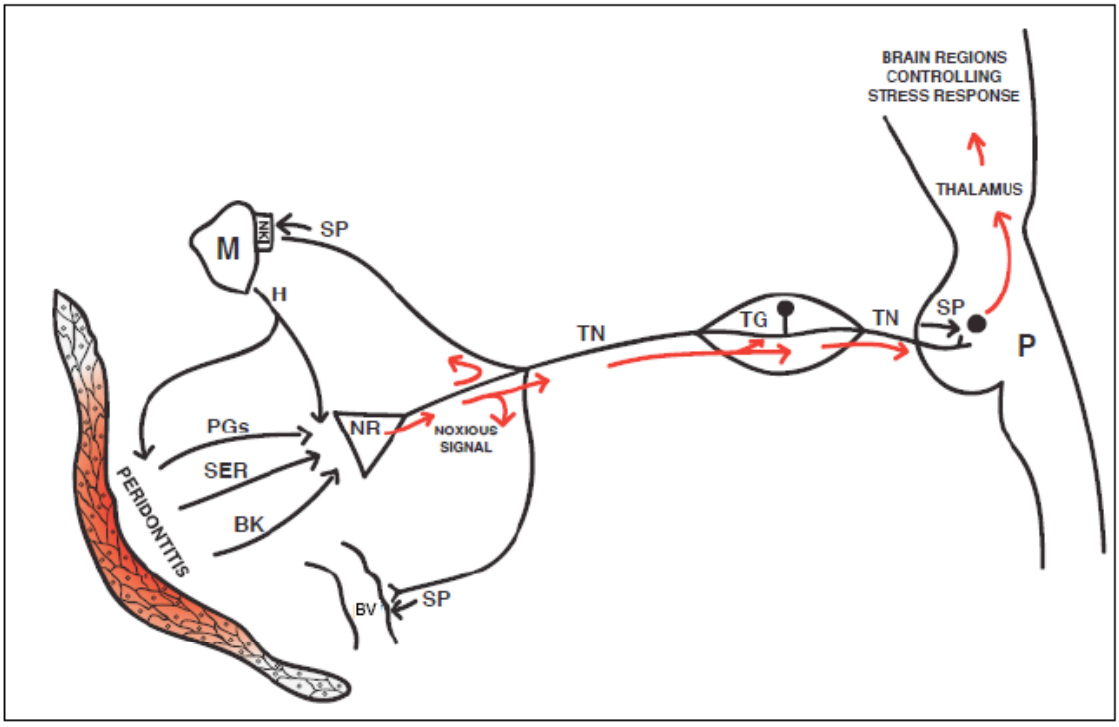

Fig. (2). Peripheral/periodontal signaling and it projection to the brain. Tissue damage causes the secretion of chemical substances which activate peripheral nociceptors (NR) and thus, generating signals that lead to the release of substance P (SP). The signals also project into the brain stem ascending by trigeminal nerve (TN) through the trigeminal ganglion (TG) vía pons (P) and from there to thalamus, then into brain centers contributing to the stress response. Prostaglandins (PGs), serotonin (SER), bradykinin (BK), mast cell (M), neurokynin 1 receptor (NK1), histamine (H), blood vessel (BV). Modified from Bear *et al.* [34].

the central nucleus. Importantly, the amygdala and hypothalamus are connected by the stria terminalis (ST). Fibers of the ST arch along the border of the caudate nucleus and the thalamus and terminate in the bed nucleus of the ST. A portion of the postcommissural portion of the ST terminates in the anterior hypothalamic area, while some of the precommissural fibers of the ST end in the basal hypothalamus. The amygdala, ST and the hypothalamus are all rich in substance P and both the amygdala and hypothalamus play important roles in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, which is associated with the central stress response. Critical to activation of the central stress response is the stimulation of hypothalamic corticotrophin releasing hormone (CRH). The release of CRH subsequently induces the secretion of adrenocorticotrophin hormone (ACTH) from the anterior pituitary, which in turn causes the release of cortisol, a glucocorticoid produced by the adrenal gland. Interestingly, activation of the central amygdaloid nucleus leads to the stress response, and it is known that substance P stimulates the HPA axis by stimulating the hypothalamic CRH neurons. Thus, it is clear that in addition to the peripheral response to the noxious stimuli as discussed above, there is also a central response to activate the stress axis; hence, further complicating periodontal recovery processes [36, 37].

As stated above, the activation of the central stress response depends on the brain receiving and integrating a great diversity of neurosecretory and blood-borne signals that arrive through distinct pathways. Thus, in addition to the peripheral responses and subsequent noxious signals transferred to and ascending within the CNS to areas responsible for initiating the basic stress response, there are also others neural components that contribute to stress responses. In this regard, vasopressin (VP), also produced in the hypothalamus, is released together with CRH during stress and acts as a potent synergistic factor of CRH on ACTH release. Also, it is well known that there are multiple interactions between the various components of the stress response. There are connections between the hypothalamic-CRH and brainstem-norepinephrine (NE) neurons, with CRH and NE capable of stimulating each other. Also, they receive stimulatory innervations from serotonergic and cholinergic systems, and inhibitory inputs from gamma-aminobutyric acid, benzodiazepine, opioids and glucocorticoids. All of these interactions are complex and non-linear, meaning that when any mediator is increased or decreased there are compensatory changes in the other mediators that depend on the time course and level of change of each of them [38, 39].

CROSSTALK BETWEEN THE CNS AND THE IMMUNE SYSTEM DURING INFLAMMATION

We just described the crosstalk between gingiva and brain with emphasis in SP and stress. During inflammation, immune cells synthesize and release cytokines which act on the target tissues and also activate the HPA axis and other components of the stress system. Reciprocally, corticosteroids and catecholamines, through their receptors, act on specific immune cells. The presence of glucocorticoid receptors (GR) and adrenergic receptors have been found on almost all immune cells. Cytokines can reach the brain through the fenestrated endothelia after crossing the blood brain barrier o fcircumventricular organs such as the organum vasculosum of the lamina terminalis. Furthermore, it has been shown that hypothalamic neurons and glial cells synthetize IL-1 and when activated by antigens, the cytokine is released and acts locally. In the periphery, cytokines can modify directly the function of sensory and sympathetic nerves, as well as the expression of adrenergic and hormonal receptors on immune cells [40].

It has been shown that there is an activation of the stress response by pro-inflammatory cytokines. The Th-1 cytokines, such as IL-1, TNF-alpha and IL-6 tend to protect against periodontal breakdown, whereas the Th-2 cytokines, such as IL-4, IL-10 and IL-23 tend to increase periodontitis. During stress the healing process is difficult due to changes in the host response. It has been demonstrated by numerous authors that stress delays healing. This is supported by a recent article showing that stress impairs bacterial clearance due to alterations in the phagocytic abilities of neutrophils and macrophages [41].

The periodontal pathogens form a microbial community in a biofilm where they can communicate between themselves [42] and with the host by signaling molecules [43]. Furthermore, there also exists a signaling between the host and some pathogens. Specifically, by host produced epinephrine (E) and norepinephrine (NE) through the newly identified bacterial adrenergic receptors. Therefore, catecholamines can increase the number of aerobic and facultative anaerobic bacteria, but in some cases could also increase virulence [44]. Another host molecule that has an effect on bacterial virulence is the opioid hormone,dynorphin [45]. These findings suggest that during stress the increased levels of epinephrine in saliva can stimulate directly the increase in the number of some bacteria in periodontitis [46].

It is well known that stimulation of the HPA axis increases the release of glucocorticoids (GC). The ability of GC to interact with immune cells is dynamically regulated and impairment in the HPA axis can lead to hipercorticolism or to glucocorticoid resistance with enhanced inflammatory state. Since the GC action is through its receptors (GR), it has been shown that the increased expression of the GR beta isoform (an inactive form), relative to GR alpha isoform (a pro-inflammatory isoform), can lead to GC resistance [47]. Furthermore, it has been shown that pro-inflammatory cytokines regulate GR gene expression and induce accumulation of the dominant negative beta isoform [48]. Environmental factors such as chronic inflammation and chronic stress, as well as epigenetic changes in GR gene have also been described [49].

Neural circuitry can be activated by stress and emotions as influenced by inflammation. The activation of HPA axis and the sympathomedular axis (SMA) in response to systemic stressors is a result of specific brain pathways. In contrast, the activation in response to emotional stressors involves a wide range of brain regions such as amygdala, the lateral septum, hippocampus, medial prefrontal cortex, bed nucleus of the stria terminalis and some hypothalamic nuclei. Emotional stressors release not only catecholamines and HPA hormones but also prolactin, glucose and induce changes in food intake as well as gastric ulceration [50]. The output is through the HPA axis and the descending autonomic nervous system. It has been shown that psychological stress or emotions alone can activate the substance P system, triggering inflammation [51].

Inflammatory processes can activate the sensory afferent to central nucleus of amygdala which has been shown to mediate behavioral symptoms of illness. Furthermore, substance P could mediate some of these behavioral symptoms. It is known that sickness behavior consists of symptoms such as weakness, malaise, difficulty concentrating, lethargy and a depressive mood which can be mediated by the action of pro-inflammatory cytokines, particularly IL-1 beta and TNF-alpha, IL-6 [52].

In several studies it has been documented that psychological stress can directly affect periodontitis. An early study showed a relationship of periodontitis with an inadequate coping with stress [53]. Another study reported that patients with chronic periodontitis differed statistically from control group without periodontitis as revealed by a psycho diagnostic survey on stress coping strategies. The results showed that periodontitis patients differ from controls when active coping strategies were used compared to patients that used distractive coping strategies. Also, differences were observed when they used defensive coping or aggression, and/or the use of pharmaceutical drugs. Furthermore, patients with defensive coping style presented a greater attachment loss. These individual behaviors in face of stressfull conditions are considered dependent on the previous experiences and genetic constitution of each person [54].

The latest discoveries show that the neuropeptide oxytocin (OT) plays a role in behavior and stress response. The peptide oxytocin is mainly synthesized and released from magnocellular neurons from paraventricular nucleus (PVN) and supraoptic nucleus (SON).The major part is transported to the posterior pituitary where it reaches the circulation and acts peripherally. The rest is transported by dendrites to different parts of the brain where exerts its multiple behavioral effects. The role of OT in maternal care and bonding has been the focus of most studies. As a curiosity, it has been shown that dog owners that have a high degree of bonding to their dog, have urinary OT higher levels after interacting with his pet [55]. Another effect is on "trust". Trust is essential for appropriate social interactions, being it a new friend or possible bussines partner. When the plasma OT is in upper level, it enhances the individual propensity to trust. Only happens when the other person does not exhibit threatening signals, by body language or face expressions [56, 57].

Oxytocin is also released during stress and contrary to CRH and VP acts to diminish the activation of HPA axis. This action is mediated within the PVN, where exists synaptic contacts between oxytocin receptors expressed on CRH neurons [58]. Oxytocin not only acts as buffer on HPA axis it also exerts anxiolytic effects since there is a high density of OT and OTR in amygdala. OT also promotes immune defense [59, 60]. Several molecular genetic studies have shown an association between oxytocin receptors (OTR) and general socio behavioral phenotypes. Recently it was shown that a genetic polymorphism of the gen for OTR was associated with the volumetric decrease of amygdaloid nucleus and an increase in its activity. This phenomenon could be related to a vulnerability to stress and depression [61, 62]. Conversely, genetic studies have shown a protective effect on disposition as well as physiological stress. These studies suggest that genetic variation of the OTR could explain partially the different coping mechanisms to stress observed in periodontal patients [63].

ENDOCANNABINOIDS AND PERIODONTAL DISEASE

Although cannabis is known to have been used over millenia for its mind altering effects as well as its medical properties, it was only after the isolation and structure elucidation of tetrahydrocannabinol (THC) it was revealed to be the active principle of marijuana by Raphael Mechoulam in 1964 [64] and shortly after synthesized [65] thousands of papers have been published. After about 20 years it was reported the existence of cannabinoid binding sites in the brain [66] and followed by the cloning of the CB_1 receptor [67]. Subsequently, a second receptor CB_2 was identified. They both belong to the superfamily of Gi/o protein receptors and both coupled to adenylyl cyclase (which they inhibit) and to mitogen-activated protein kinases (MAPK). They also regulate ion channels, such as Ca^{++} and K^{+} ion channels. The endogenous cannabinoids are naturally occurring substances that bind to cannabinoid receptors and elicit similar actions to THC. They are members of the eicosanoid superfamily that derive from arachidonic acid and are produced "on demand" in many different organs and tissues. The first endocannabinoid to be discovered was "anandamide" (AEA) [68] followed by 2-arachidonylglycerol (2-AG). CB_1 receptor is the major cannabinoid receptor in the brain in neuronal cells. It also occurs in endocrine cells and others peripheral tissues. In the brain, this receptor presents high density in basal ganglia, substancia nigra, globus pallidus, hippocampus and cerebellum. The endocannabinoids have important role in motivation and cognition, as well as in GABAergic and glutamatergic neurotransmission, acting as inhibitors of neurotransmission release. One of the functions of endocannabinoids is to modulate hypothalamic-pituitary-adrenal

axis and to reestablish homeostasis when is altered by stress. Also, endocannabinoids play an important role as anxiolytic [69, 70].

There are many reports on the effects of endocannabinoids in many inflammatory diseases [71]. It was reported the presence of AEA in human gingival tissue and that AEA regulates the periodontal inflammation through inhibition of the NF-kB pathway [72]. Recently, we have reported that local injection of AEA into gingiva of rats showing experimental periodontitis and submitted to immobilization stress produced a lowering in TNF-alpha and IL-1 beta content in the gingiva. Those inhibitory effects on these pro-inflammatory cytokines were mediated by cannabinoid receptors CB_1 and CB_2, since the injection of antagonists to both receptors prevented this decrease. Therefore, we concluded that endocannabinoid AEA diminishes the inflammatory response in periodontitis, even during a stressful situation [73]. Other investigators, observed that after a month of treatment with cannabidiol (a non-psychomimetic constituent of cannabis) rats with experimental periodontitis showed decreased TNF-alpha and IL-1 beta as well as a decrease in bone resorption due to inhibition of RANK/RANKL expression [74]. Another report showed that human periodontal ligament (hPDL) cells express CB_2 receptors. The authors used an agonist to CB_2 receptors (HU-308) and found that it enhanced the mRNA levels of the osteogenic genes in hPDL cells such as Runx2, OC, and APL. Furthermore, the OPG mRNA was increased and RANKL mRNA decreased. The authors concluded that endocannabinoids play an important role in alveolar bone metabolism [75]. This opens new possibilities for investigations of new pharmacological approaches to treat periodontal disease.

DIABETES AND PERIODONTAL DISEASE

Besides stress, another risk factor recognized for periodontitis is diabetes [76]. Recent papers show that there is a "two-way" relationship between diabetes and periodontal disease [77, 78]. It has been demonstrated that severe periodontal disease has a negative effect on glycemic control in diabetics [79] and development of nephropathies as other complications in patients with type 2 diabetes [80]. The inflammation is considered to be a part of pathogenesis of diabetes and chronic periodontitis. This inflammatory state in diabetes type 1 and type 2, with elevated serum markers of inflammation such as TNF-alpha, IL-6 and C-reactive protein, can contribute to insulin resistance, microvascular and macrovascular complications and hyperglicemia that increase even more inflammation and oxidative stress [81]. It has been reported that diabetes increases gingival crevicular fluid levels of PGE2 and IL-1 beta in periodontitis [82]. On the other hand, periodontal treatment reduces not only serum glucose levels but also lipids and even blood pressure in diabetic patients [79].

Since the bacterial biofilm does not differ between patients with periodontal disease with or without diabetes, therefore is the host response that must be different and responsible of this hyperinflammation in patients with chronic periodontitis and diabetes.They present monocyte phenotypes with enhanced levels of pro-inflammatory cytokines. The role of the receptor for the advanced glycation end products (RAGE), a multiligand signaling receptor and member of immunoglobulin superfamily of cell-surface molecules, is increased in diabetic patients and its activation through interaction with its ligands (AGEs) plays a role in complications such as cardiovascular and kidney diseases in diabetic patients. The expression of RAGE and AGEs and of markers of oxidative stress were found in gingiva of patients with diabetes and periodontal disease. Also, AGEs could be measured in serum of these patients. The accumulation of AGEs and their interaction with RAGE is accompanied by tissue destructive matrix metaloproteinases in gingival tissue of these patients. Also, they inhibit the expression of collagens type I and III in human gingiva. Furthermore they contribute to osteoclastogenesis via increased expression of RANKL and decrease in OPG [77].

It is not only diabetes type 2 that increases the risk of periodontal disease but also type 1, therefore all patients with diabetes, including children and young adults, should be considered at increased risk of periodontal disease. We strongly recommend to physicians that treat patients of all ages with diabetes to be aware of the possible presence of periodontal disease and to dentists that treat patients with periodontitis to check if the patients have diabetes.

CARDIOVASCULAR DISEASE AND PERIODONTAL DISEASE

Local infections can initiate chronic inflammatory processes that may cause systemic or organic specific diseases elsewhere in the body. After the appearance of two pioneering studies on association of dental infection and myocardial infarction [83] and cerebral infarction [84] many studies confirmed the hypothesis that there is a causal connection between periodontal disease and cardiovascular disease (CVD), for details see [85]. The CVD compromise a group of conditions such as coronary heart disease, congestive heart failure, myocardial infarction, stroke and hypertension whose etiological shared factor is atherosclerosis. Atherosclerosis is considered an inflammatory and metabolic condition with lipid accumulation [86]. The entry of bacteria and its products into circulation is considered to be the initiation of this process. Since transient bacteremia occurs in patients with periodontal disease as well as tonsillitis, pharyngeal infections and some processes of the gut. These bacterias, are also frequently found in the atherosclerotic specimens [87-89]. Furthermore, the DNA of P.g and of A.a has been isolated. Only one study found a living P. g in atherosclerotic sample [90].It is considered that the amount of bacterial DNA correlates with the number of local leucocytes, inferring the influence of bacterial load on atherosclerotic inflammation. There is also a correlation between increased atherosclerotic biomarker levels and oral and gut microbiota abundance [88].

The two bacteria that are found in periodontal disease and also in the blood and its DNA in atherosclerotic plaques are P.g and A.a. The P.g major virulence factors are gingipains (tripsin-like cysteine proteinases) and of A.a is a potent leukotoxin. The P.g binds through its fimbriae to the endothelial cells, whose respond by secreting pro-atherogenic mediators. In a very important study by Belstrom *et al*. [90] the authors showed that P.g adheres to red blood cells and therefore it's binding to neutrophils and monocytes is reduced and this way the bacteria evades the phagocytic cells and furthermore uses the red blood cells as transport. The same was found also for A.a. Another factor that plays an important role in atherosclerosis is lipid metabolism. It is also a very important risk factor for the development of CVD. It has been shown that periodontal disease presents an increase in plasma cholesterol, LDL and triglycerides. It was suggested that this could be due to pro-inflammatory cytokines. There are some markers associated with vascular events that include cholesterol, LDL, HDL, lipoprotein, homocysteine, IL-6 and the most used C-reactive protein [91].

STROKE AND PERIODONTAL DISEASE

Cerebrovascular disease is among the most prevalent causes of death and disablement in industrialized countries. Recent findings suggest that atherogenesis and plaque rupture are two factors of cerebrovascular pathogenesis that result from systemic and vascular inflammatory processes which are due to chronic infections such as periodontal disease. Using a meta-analysis of the English literature an association of periodontal disease with increased risk of stroke was found [92]. Also, a positive correlation between probing depth and the increased levels of P.g DNA in the subgingival plaque of patients with periodontal disease and stroke was reported [93]. These findings show that periodontal disease is a risk factor for the development of cerebral hemorrhage or infarction. Therefore, an

early treatment of chronic periodontitis may counteract the development of cerebrovascular episodes.

METABOLIC SYNDROME AND PERIODONTAL DISEASE

Metabolic Syndrome is a clinical entity that encompasses several risk factors for CVD. It consists of impaired glucose regulation, abdominal obesity, dyslipidemia and high blood pressure [94]. It is estimated that around a quarter of world´s adult population presents metabolic syndrome [95]. The etiology is a "pro-inflammatory" state due to excessive caloric intake and other chronic inflammatory conditions. This pro-inflammatory state leads to an increase in oxidative stress. Oxidative stress is a persistent imbalance between the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and anti-oxidant defenses. There is increasing evidence linking periodontal disease to systemic disease, such as diabetes, rheumatoid arthritis and CVD. A potential factor which could increase insulin resistance is the production of ROS. Furthermore it was also found an increase in oxidative stress with increase in ROS in gingiva of patients with periodontal disease [96]. Therefore, it is possible that oxidative stress could be the link between metabolic syndrome, periodontal disease, diabetes and cardiovascular diseases.

CONCLUSIONS

It is evident that the interactions between the nervous, endocrine and immune system influence each other. In addition, the psychosocial factors can induce an alteration of the equilibrium between these systems and negatively influence the evolution or complicate these already very complex diseases. Furthermore, the entry of oral gram negative bacteria and inflammatory products into general circulation of chronic periodontitis is well documented. On the other hand, the meta-analysis studies all over the world show statistically significant relations between periodontal disease and cardiovascular diseases, and with diabetes and metabolic syndrome.

All these facts must be taken as a warning to all of us about the higher risk to have a stroke or myocardial infarction if we have chronic periodontitis.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This research was partially supported by Grants from Agencia Nacional de Promoción Científica y Tecnológica PICT 06-0258 and Consejo Nacional de Investigaciones Científicas y Técnicas PIP 02546.

We want to express our gratitude to Hugo A. Araujo for his dedicated revision of the manuscript (C. W. Bill Young Marrow Donor Program, Georgetown University, Washington D.C. USA) and to Dr Fernando G. Correa for his comments (CEFYBO-CONICET-UBA).

REFERENCES

[1] Carranza F, Shklar G. The history of periodontology. Chicago: Quintessence 2003.

[2] Brut B, Greenwell H, Fiorellini J, *et al*. Epidemiology of Periodontal Disease. J Periodontol 2005; 76: 1406-19.

[3] Dye BA. Global periodontal disease epidemiology.Periodontology 2000 2012; 58: 10-25.

[4] Romanelli H, Gonzalez y Rivas M, Chiappe V, Gomez M, Macchi R. Periodontal treatment needs in Argentine adult subjects. Acta Odontol Latinoam 2007; 20: 39-47.

[5] Loos BG, van der Velden U, Laine ML. Susceptibility. In: Lindhe J, Lang NP, Karring T, Eds. Clinical Periodontology and Implant Dentistry. Iowa: Blackwell Minksgaard 2008; pp: 328-46.

[6] Scantamburlo G, Scheen AJ. Role du stress psycho-social dans les maladies complexes. Rev Med Liege 2012; 67: 234-42.

[7] Davanian H, Stranneheim H, Bage T, *et al*. Gene expression profiles in paired gingival biopsies from periodontitis-affected and healthy tissue revealed by massively parallel sequencing. Plos One 2012; 7: 1-12.

[8] Peters EWJ, Liezmann C, Klapp BF, Kruse J. The neuroimmune connection interferes with tissue regeneration and chronic inflammatory disease in the skin. Ann NY Acad Sci 2012; 1262: 118-26.

[9] Bartold PM, Walsh LJ, Narayanan AS. Molecular and cell biology of the gingiva. Periodontology 2000 2000; 24: 28-55.

[10] Damek-Poprawa M, Korostoff J, Gill R, DiRienzo JM.Cell junction remodeling in gingival tissue exposed to a microbial toxin. J Dent Res 2013; 92 (6): 518-23.

[11] Caffesse RG, Nasjleti CE, Castelli WA. The role of sulcular environment in controlling epithelial keratinization. J Periodontol 1979; 50: 1-6.

[12] Hujoel PP, White BA, García RI, Listgarten MA. The dentogingival epithelial surface area revisited. J Periodont Res 2001; 36: 48-55.

[13] Tsukamoto Y, Usui M, Yamamoto G, *et al*. Role of the junctional epithelium in periodontal innate defense and homeostasis. J Periodontol Res 2012; 47: 750-57.

[14] Fiorellini JP, Kim DM, Ishikawa SO. The Gingiva. Normal Periodontium. In: Carranza FA Ed. Carranza's Clinical Periodontology. St. Louis, Missouri. Saunders Elsevier 2006; pp: 46-67.

[15] Lindhe J, Karring T, Araujo M. The anatomy of periodontal tissues. Anatomy. In: Lindhe J, Lang NP, Karring T, Eds. Clinical Periodontology and Implant Dentistry. Oxford UK: Blackwell Munksgaard 2008; pp: 3-49.

[16] Gyorfi A, Fazekas A, Rosivall L. Neurogenic inflammation and the oral mucosa. J Clin Periodontol 1992; 19: 731-36.

[17] Newman MG. The Normal Periodontium.Blood Supply, Lymphatics and Nerves. In Carranza's FA, Ed. Clinical Periodontology. Saunders Elsevier. Missouri 2006; pp: 59-61.

[18] Iwasaki K, Komaki M, Yokoyama N, *et al*. Periodontal ligament stem cells possess the characteristics of pericytes. J Periodontol 2012; Dec 14 Epub.

[19] Liu D, Xu J, Liu O, *et al*. Mesenchymal stem cells derived from inflamed periodontal ligament exhibit impaired immunomodulation. J Clin Periodontol 2012; 39: 1174-82.

[20] Park JC, Kim JM, Jung IH, *et al*. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: *in vitro* and *in vivo* evaluations. J ClinPeriodontol 2011; 38: 721-31.

[21] Liang S, Hosur KB, Domon H, Hajishengallis G. Periodontal inflammation and bone loss in aged mice. J Periodontal Res 2010; 45: 574-78.

[22] De la Fuente M. Role of neuroimmunomodulation in aging. Neuroimmunomodulation 2008; 15: 213-23.

[23] Berglundh T, Donati M. Aspects of adaptative host response in periodontitis. Proceedings from the 5th European Workshop of Periodontology. J Clin Periodontol 2005; 32: 87-107.

[24] Offenbacher S. Maternal periodontal infections, prematurity, and growth restriction. Clin Obstet Gynec 2004; 47: 808-21.

[25] Mahanonda R, Pichyangkul S. Toll-like receptors and their role in periodontal health and disease. Periodontol 2000 2007; 43: 41-55.

[26] Beklen A, Hukkanen M, Richardson R, Konttinen YT. Immunohistochemical localization of toll-like receptors 1-10 in periodontitis. Oral Microbiol Immunol 2008; 23: 425-31.

[27] Mayank Hans, VeenuMadaan Hans. Toll-like receptors and their dual role in periodontitis: a review. J Oral Sci 2011; 53: 263-71.

[28] Kirkwood KL, Taba M Jr, Rossa C, *et al*. Molecular biology of the host-microbe interaction in periodontal diseases: Selected topics: Molecular signaling aspects of pathogen-mediated bone destruction in periodontal disease. In: Carranza FA, Eds. Carranza's Clinical Periodontology. Saunders-Elsevier 10th edit 2006; pp: 259-82.

[29] Breivik T, Sluyter F, Hof M, Cools A. Differential susceptibility to periodontitis in genetically selected Wistar rat lines that differ in their behavioral and endocrinological response to stressors. Behavioral Genetics 2000; 30: 123-30.

[30] Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. J Clin Periodontol 2012; 39: 239-48.

[31] Selye H. A syndrome produced by diverse nocuous agents. Nature 1936; 138: 32

[32] Selye H. The general adaptation syndrome and the diseases of adaptation. J Clin Endocrinol 1946; 6: 117-30.

[33] Selye H. Stress and disease. Science 1955; 122: 625-31.

[34] Bear MF, Connors BW, Paradiso MA. Neuroscience: Exploring the Brain. 3rd ed. Philadelphia: Lippincott 2006.
[35] Fristad I, Vandemska-Radunovick K, Fjild k, Wimalawansa SI and Kvinnsland IH. NBK1, NK2, NK3 and CGRP1 receptors identified in rat oral soft tissues and in bone and dental hard tissue cells. Cell Tissue Res 2003; 311: 383-91.
[36] Dees WL, Kozlowski GP. Effect of castration and ethanol on amygdaloid substance P immunoreactivity. Neuroendocrinology 1984; 39: 231-35.
[37] O´Connor TM, O´Halloran DJ and Shanahan F. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. Q J Med 2000; 93: 323-33.
[38] Chrousos GP. Stressors, stress and neuroendocrine integration of the adaptive response. In: Stress of life from molecules to man. Czermely P. Edit. Ann New York Acad Scie. New York, 1998; 851: 311-35.
[39] McEwen BC. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol 2008; 583: 174-85.
[40] Dantzer R, O´Connor J, Freund GG, Johnson RW and Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosc 2008; 9: 46-57.
[41] Tymen SD, Rojas IG, Zhou X, Fang ZJ, Zhao Y, Marucha PT. Restrain stress alters neurophil and macrophage phenotypes during wound healing. Brain Behav Immun 2012. pii: S0889-1591(12)00196-1. doi: 10.1016/j.bbi.2012.07.013.
[42] Samuel BS, Gordon JI. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. Proc Natl Acad Sci USA 2006; 103: 10011-6.
[43] Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122: 107-18.
[44] Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. The QseC sensor kinase: a bacterial adrenergic receptor. Proc Natl Acad Sci USA 2006; 103: 10420-26.
[45] Zaborina O, Lepine F, Xiao G *et al.* Dynorphin activates quórum sensing quinolone signaling in Pseudomonas aeruginosa. PloS Pathog 2007; 3: e35.
[46] Akcali A, Huck O, Tenenbaum H, Davideau JL, Buduneli N. Periodontal diseases and stress: a brief review. J Oral Rehabil 2012; 40:60-8.
[47] Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Ann N.Y. AcadSci 2012; 1261: 55-63.
[48] Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci USA 2001; 98: 6865-70.
[49] McGowan PO, Sasaki A, D'Alessio AC, *et al.* Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 2009; 12: 342-38.
[50] Armario A, Davin N, Muñoz-Abellan C, *et al.* What can we know from pituitary-adrenal hormones about the nature and consequences of exposure to emotional stressors? Cell Mol Neurobiol 2012; 32: 749-58.
[51] Erin N, Ersoy Y, Ercan F, Akici A, Oktay S. NK-1 antagonist CP99994 inhibits stress-induced mast cell degranulation in rats. Clin Exper Dermatol 2004; 29: 644-8.
[52] Dantzer R, and Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain, Behavior Immunity 2007; 21: 153-60.
[53] Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA. Relationship of stress, distress, and inadequate coping behaviors to periodontal disease. J Periodontol 1999; 70: 711-23.
[54] Wimmer G, Janda M, Wieselmann-Penkner K, Jakse N, Polansky R, Pertl C. Coping with stress: Its influence on periodontal disease. J Periodontol 2002; 73: 1343-51.
[55] Nagasawa M, Kikusui T, Onaka T, Ohta M. Dog´s gaze at his owner increases owners urinary oxytocin during social interaction. Horm Behav 2009; 55: 434-41.
[56] Zak P. The neurobiology of trust. Sci Am 2008; 298: 88-95.
[57] Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Ferh E. Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron 2008; 58: 639-50.
[58] Dabrowska J, Hazra R, Ahern TH, *et al.* Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin system in the hypothalamus and the bed nucleus of the stria terminalis of the rat: implications for balancing stress and affect. Psychoneuroendocrinology 2011; 36: 1312-26.
[59] Lee HJ, Macbeth AH, Pagana JH, Scott Young 3rd W. Oxytocin: The great facilitator of life. Prog Neurobiol 2009; 88: 127-51.
[60] Smith AS, Wang Z. Salubrious effects of oxytocin on social stress-induced deficits. Hormones Behavior 2012; 61: 320-30.
[61] Furman D, Chen M, Gotlib I, *et al.* Variant in oxytocin receptor gene is associated with amygdale volume. Psyconeuroendocrinology 2011; 36: 891-7.
[62] Shinozaki G. The integrated model of serotonin transporter gene variation (5HTTLPR) and the glial cell transporter in stress vulnerability and depression. Med Hypotheses 2012; 78: 410-14.
[63] Rodrigues SM, Saslow IR, Garcia N, John OP, Keltner D. Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. Proc Natl Acad Sci USA 2009; 106: 21437-41.
[64] Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. J Am ChemSoc 1964; 86: 1646-47.
[65] Mechoulam R, Braun R, Gaoni Y. A stereospecific synthesis of (-) delta 1 and (-) delta 6 –tetrahydrocannabinols. J Am Chem Soc 1967; 89: 4552-54.
[66] Devane WA, Dysarz FA 3rd, Johnson MR, Melvin IS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988; 34: 605-13.
[67] Matsuda LA, Lolair SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990; 346: 561-64.
[68] Devane WA, Hanus I, Breuer A, *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992; 258: 1946-49.
[69] Riebe CI, Wotjak CT. Endocannabinoids and stress. Stress 2011; 14: 384-97.
[70] Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annu Rev Psychol 2013; 64: 6.1-6.27.
[71] Burstein SH, Zurier R. Cannabinoids, Endocannabinoids and related analogs in inflammation.The AAPS Journal 2009; 11: 109-11.
[72] Nakajima Y, Furuichi Y, Biswas KK, *et al.* Endocannabinoid, anandamide in gingival tissue regulates the periodontal inflammation through NF-kB pathway inhibition. FEBS Letters 2006; 580: 613-9.
[73] Rettori E, De Laurentiis A, Zubilete-Zorrilla M, Rettori V, Elverdin JC. Anti-inflammatory effect of the endocannabinoid anandamide in experimental periodontitis and stress in the rat. Neuroimmunomodulation 2012; 19: 293-303.
[74] Napimoga MN, Benatti BB, Lima FO, *et al.* Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. Int Immunopharmacol 2009; 9: 216-22.
[75] Qian H, Zhao Y, Peng Y, *et al.* Activation of cannabinoid receptor CB2 regulates osteogenic and osteoclastogenic gene expression in human periodontal ligament cells. J Periodont Res 2010; 45: 504-11.
[76] Chavarry NGM, Vettore MV, Sansone C, Sheiham A. The relationship between diabetes mellitus and destructive periodontal disease: a meta-analysis. Oral Health Prev Dent 2009; 7: 107-27.
[77] Preshaw PM, Alba AL, Herrera D, *et al.* Periodontitis and diabetes: a two-way relationship. Diabetologia 2012; 55: 21-31.
[78] Lalla E, and Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. Nat Rev Endocrinol 2011; 7: 738-8.
[79] Teeuw WJ, Gerdes VF, Loos BG. Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. Diabetes Care 2010; 33: 421-27.
[80] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005; 54: 1615-25.
[81] MealeyBL, and Oates TW.Diabetes Mellitus and Periodontal Disease. J Priodontol 2006; 77: 1289-303.
[82] Engebretson SP, Hey-Hadavi J, Ehrhardt FJ *et al.* Gingival crevicular fluid levels of IL-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. J Periodontol 2004; 75: 1201-8.

[83] Mattila KJ, Nieminen VV, Valtonen VP, *et al*. Association between dental health and acute myocardial infarction.BMJ 1989; 298: 779-81.

[84] Syrjanen J, Peltola J, Valtonen V, Iivanainen M, Kaste M, Huttunen JK. Dental infections in association with cerebral infarction in Young and middle-aged men. J intern Med 1989; 225: 179-84.

[85] Belstrom D, Damgaard C, Nielsen CH, Holmstrup P. Does a causal relation between cardiovascular disease and periodontitis exist? Microbes Infection 2012; 14: 411-8.

[86] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105: 1135-43.

[87] Inaha P, Amano A. Roles of oral bacteria in cardiovascular diseases – from molecular mechanisms to clinical cases implication of periodontal diseases in development of systemic diseases. J Pharmacol Sci 2010; 113: 103-9.

[88] Koren O, Spor A, Felin J, *et al*. Human oral, gut and plaque microbiota in patients with atherosclerosis.Pro cNatl Acad Sci USA 201;108:4592-8.

[89] Kebschull M, Demmer RT, Papapanou PN. "Gum bug, leave my heart alone!" –epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. J Dent Res 2010; 89: 879-902.

[90] Belstrom D, Holmstrop P, Damgard C, *et al*. Atherogenic bacterium Porphyromonasgingivalis evades circulating phagocytes by adhering to erythrocytes. Infect Immun 2011; 79: 1559-65.

[91] Ridker PM, and Silvertown JD. Inflammation, C-Reactive Protein, and atherothrombosis. J Periodontol 2008; 79: 1544-51.

[92] Sfyroeras GS, Roussas N, Saleptsis GV, Argyriou C, Giannoukas D. Association between periodontal disease and stroke. J Vasc Surg 2012; 55: 1178-84.

[93] Ghizoni JS, Taveira LA, Garlet GP, *et al*. Increased levels of *Porphyromonas gingivalis* are associated with ischemic and hemorrhagic cerebrovascular disease in humans: an *in vivo* study. J Appl Oral Sci 2012; 20:104-12.

[94] Eckel RH, Grundy SM, Zimmet PZ. The Metabolic Syndrome. Lancet 2005; 365: 1415-28.

[95] Cameron AJ, Show JE, Zimmet PZ. The metabolic syndrome prevalence in world wide population. Endocrinol Metab Clin North Am 2004; 33: 351-75.

[96] Bullon P, Morillo JM Ramirez-Tortosa MC, *et al.* Metabolic Syndrome and periodontitis: Is oxidative stress a common link? J Dent Res 2009; 88: 503-18.

Received: September 24, 2013 **Accepted: January 29, 2014**