

## Immune Response in Periodontitis and Modulatory Value of Staphylococcal Antigens

Grigore MIHAESCU<sup>1</sup>, Paula PERLEA<sup>2</sup>, Dorin MERCUT<sup>3</sup>, Corneliu Ovidiu Vrancianu<sup>1,6</sup>, Roxana Elena CRISTIAN<sup>4</sup>, Madalina PREDA<sup>5</sup>, Mariana Carmen CHIFIRIUC<sup>1,6\*</sup>, Marian Vladimir CONSTANTINESCU<sup>7</sup>, Lakshman Perera SAMARANAYAKE<sup>8</sup>, Irina CODITA<sup>9</sup>, Teodor GEORGESCU<sup>10</sup>

<sup>1</sup>University of Bucharest, Faculty of Biology, Microbiology-Immunology Department; ovidiu.vrancianu@yahoo.com; carmen.chifiriuc@bio.unibuc.ro; grigoremihaescu2006@yahoo.com; roxana.cristian95@yahoo.com

<sup>2</sup>University of Medicine and Pharmacy "Carol Davila" Bucharest, Endodontics Department

<sup>3</sup>University of Medicine and Pharmacy Craiova

<sup>4</sup>Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, 020956 Bucharest, Romania

<sup>5</sup>University of Medicine and Pharmacy Carol Davila; madalina.prd@gmail.com

<sup>6</sup>The Research Institute of the University of Bucharest, Bucharest, Romania;

<sup>7</sup>Holistic Dental & Medical Institute of Bucharest – ROPOSTURO, Bucharest, Romania

<sup>8</sup>University of Sharjah, College of Dental Medicine, Sharjah, United Arab Emirates

<sup>9</sup>Cantacuzino National Research and Development Institute for Microbiology and Immunology, adirina\_2005@yahoo.com

<sup>10</sup>Dr. Georgescu Dental Studio; georgescuteodor@yahoo.com

Corresponding author: carmen.chifiriuc@bio.unibuc.ro

**Abstract.** Periodontal disease is a plaque microbiome related, that disrupts the homeostasis of the periodontium and the supporting alveolar bone. The aim of this brief review is to offer the reader the theoretical background of the current status of immunomodulatory therapies used in the management of periodontitis using staphylococcal antigens. In response to the hypothesis that *Staphylococcus aureus* helps ameliorate periodontal disease, the National Development Institute for Microbiology and Immunology Cantacuzino, Romania, produced the “Staphylococcal D Vaccine”. The proponents of this product claim that Staphylococcal Vaccine is highly efficacious in the management of periodontitis particularly in patients with clinical signs of edema, papillary hypertrophy, bleeding on probing, and pockets > 7 - 8 mm in depth. Although the mechanism of action of staphylococcal antigen vaccines is elusive as yet, it appears that such vaccination leads to a dramatic reduction in periodontal inflammation, and periodontal pocket depth. However, more research is required to obtain conclusive data on the subject.

**Keywords:** *Staphylococcus*; immune response; periodontitis; inflammation; modulation

## 1. Introduction

A dysbiotic oral microbiome and the associated plaque biofilms are generally considered to be the major agents of the chronic inflammatory process that often leads to periodontal inflammation. Dental plaque biofilms on tooth surface is formed by numerous bacterial species comprising the oral microbiota. Only about 60% of the microbial species of the biofilm microbiota can be identified by the classical methods based on culturable flora, but the novel molecular advances such as the new generation sequencing (NGS) technology have revealed an array of novel organisms that inhabit the gingival crevice [1]. All these biofilm organisms including bacteria, fungi and archaea, use complex metabolic processes and cellular interactions, and they constitutes a very balanced, ecosystem living in harmony with the host [2]. However, when such a symbiotic system is disrupted leading to dysbiosis, the biofilm flora cause pathology of the anchoring tissue of the tooth including the periodontal membrane and the alveolar bone.

## 2. Dental plaque formation

Initially, within a few minutes, after cleaning a tooth surface, the microbial colonization process ensues with the arrival of pioneer bacterial, the streptococci which adhere to the salivary pellicle. Subsequently, within a few hours the bacterial community is colonised by secondary arrivals such as protease producing *Actinomyces* spp [3]. There is also a concomitant development of the characteristic biofilm matrix of extracellular polysaccharides which in turn favors other colonizers such as *Capnocytophaga* sp., *Eikenella* sp., *Haemophilus* sp., *Prevotella* sp., *Veillonella* spp. Subsequently, the structure of the community becomes further complicated by the arrival of the late colonizers which need an anaerobic ecosystem to thrive. These are organisms such *Treponema denticola*, *Porphyromonas gingivalis* and *Tannerella forsythia* which play critical role in periodontal disease intuition and progress.

The bridge and the skeletal structure between the early and the late colonizers is the relatively larger Gram negative organism, *Fusobacterium nucleatum*, which explains their numerical abundance in normal and pathological samples. As the thickness of the biofilm increases, the oxygen concentration decreases progressively, which leads to the predominance colonization by microaerophilic and moderately anaerobic species (*Agregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Eikenella corrodens*) as well as of the strict anaerobes (*P. gingivalis*, *B. forsythus*, *Treponema denticola*, *P. intermedia*, *F. nucleatum*, *Eubacterium* sp.) [4,5]. The latter group of organisms together with *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Eubacterium*

*timidum*, *Lactobacillus sp.*, *Actinomyces naeslundii*, *Pseudomonas anaerobius*, *Eubacterium sp.*, *Bacteroides intermedius*, *Bacteroides forsythum*, *Selenomonas sputigena* and *Haemophilus aphrophilus* are characteristically isolated from deep periodontal pockets. Although most of these bacteria rarely invade the gingival tissues, they can invade the gingival epithelium and release enzymes and endotoxins that can lead to either a direct or indirect (by inducing the inflammatory process) destruction of the periodontal tissues [6-8].

In relation to the location of the plaque biofilm development, the latter can be classified as either supragingival and subgingival plaque biofilms, the latter being predominantly involved in initiating periodontal diseases.

### **2.1. Dental plaque- periodontitis relationship**

Periodontal disease (PD) is an infectious inflammatory disease that disturbs the homeostasis of its ecosystem leading to gradual injury of the gums, the alveolar bone, the periodontal ligament, and the tooth cementum [9]. The prevalence of periodontal disease increases with age, and 50% of adults are known to have some degree of periodontitis (inflammation of the gum and the tooth supporting structure) [10]. An aggressive form of the disease affects young adults and is characterized by the rapid destruction of periodontal tissue [11]. Chronic periodontal disease affects 11.2% of the world's population [12]. Chronic periodontitis is an episodic disease with progressive inflammation associated with plaque biofilm development and tartar accretion, in periodontal pockets [13,14], and alternating with periods of inflammation and remission. Interestingly the periodontal inflammation is localized, and within the same mouth, there could be some gingival sites that are uninflamed and perfectly healthy, and free of destructive periodontal disease [15]. Another major characteristic of periodontitis is the progressive inflammatory infiltration of the periodontal epithelium that also helps periodontal tissues destruction [10].

The primary etiological agent of periodontitis is the plaque biofilm that develops in the gingival crevice, and the bacterial proteolytic enzymes, and noxious metabolites that either directly or indirectly cause tissue destruction, or alternatively, impede the local host defense factors [16]. Bacterial collagenase and hyaluronidase for instance, degrade collagen and the connective tissue matrix. Furthermore, the bacterial lipopolysaccharides (LPS) or endotoxins, the major cell wall constituent of Gram-negative bacteria, released after autolysis play a critical role in the pathogenesis of periodontitis. LPS is one of the most potent stimulants of inflammation and are not neutralized by immune effectors [17].

Any condition that diminishes the efficacy of antibacterial defense mechanisms (primary or secondary immunodeficiency, diabetes, neutrophil dysfunction) predisposes an individual to periodontitis [18]. Smoking and stress are also other subsidiary risk factors for periodontal disease, and the consequent

impedance in blood flow leads to the survival of anaerobes, which in turn produce metabolites that damage the periodontal tissues.

## **2.2. The inflammatory immune response in periodontitis**

Apart from the direct effect of the bacteria and their by products on the gingival epithelium and the periodontium, the inflammatory response triggered by the plaque biofilm plays a significant role in the pathology of periodontitis [19,20]. Antigens released after lysis of Gram-negative bacteria, especially endotoxins, are potent chemical messengers for resident macrophages, which synthesize pro-inflammatory cytokines: IL-1, IL-6, and TNF (tissue necrosis factor). Interleukins, especially IL-1, induce the synthesis of other mediators such as chemotactic cytokines (IL-8), prostaglandins, and leukotrienes, that attract neutrophils and monocytes, which help maintain and amplify the inflammatory response [21,22]. The inflammatory reaction to some extent, prevents the bacterial invasion of the gingival tissue and removes bacterial byproducts, thus playing a protective role [23]. Furthermore, antibodies against bacterial cell walls, capsular material, flagellae and toxins are synthesized in the periodontium as a response to bacterial byproducts. Antibodies neutralize toxins, opsonize bacterial cells, promote phagocytosis, and activate the classical complement pathway.

## **2.3. Role of the neutrophils in the periodontal inflammatory process**

The primary actors in the pathological process leading to periodontitis are the professional phagocytes, neutrophils and macrophages. They have a limited number of pattern recognition receptors (PRRs) called pathogen-associated molecular patterns (PAMPs), that bind the pathogen antigens [24]. Neutrophils, the main effector cells of the acute inflammatory reaction, are also endowed with a wide range of bactericidal properties, the most effective of which is the myeloperoxidase (MPO)- hydrogen peroxide ( $H_2O_2$ )- halogen system [25]. After PAMP recognition, the neutrophils phagocytose the pathogen and kill the organism by both  $O_2$ -dependent and independent mechanisms. The NADPH-dependent oxidase of the neutrophil membrane is activated simultaneously and this process called the respiratory burst, in turn generates large amounts of  $O_2$  ions. The latter is rapidly converted to  $H_2O_2$  and then into other reactive molecules with a microbicidal effect: hypochlorous acid (HClO), chloramine ( $NH_2Cl$ ), hydroxyl radical, singlet oxygen. The important role of NADPH-oxidase and ROS system as anti-infectious defense system is illustrated by the chronic granulomatous disease syndrome, where patients suffer from NADPH-oxidase defect [26]. These patients have recurrent fungal and bacterial infections, particularly caused by the common skin pathogen *S. aureus*. Simultaneously, there is a production of cytoplasmic granules in neutrophils which fuse with the phagosomes. Antimicrobial peptides,  $\alpha$ -defensins, cathepsins, proteinase 3,

elastase, azurocidine are next released into the phagosome: Although the neutrophils primarily ingest and destroy the pathogens they also release enzymes into to the inflammatory focus leading to tissue injury [27]. Excessive activation of neutrophils, as well as prolongation of inflammation (3-4 days) further amplify tissue injury [28]. The increased rate of  $\beta$ -glucuronidase, myeloperoxidase, and ROS secretion which, as mentioned above, has a destructive effect on the periodontium, may explain the rapid loss of periodontal tissue in aggressive periodontitis [29].

The hyper-inflammatory phenotype of neutrophils is considered to play an important role in the pathogenesis of chronic periodontitis [30]. For instance, neutrophils derived from patients with chronic periodontitis, when exposed to *Porphyromonas gingivalis* and *A. actinomycetemcomitans*, release significantly high quantities of ROS [31].

The differentiated response of neutrophils, which leads to the activation and degranulation of the neutrophil is considered to be a preliminary priming step of the inflammatory process [32]. Such activated neutrophils have superior functional properties, hence are called hyperactive cells. The physiological state of hyperactivation is considered to be a significant factor in the destruction of periodontal tissue, especially in aggressive periodontitis [32]. The initiating agents may be circulating in the blood or are produced locally, in the inflammatory microenvironment, i.e.: circulating bacterial endotoxins, increased levels of TNF- $\alpha$ , IL-6, IFN- $\gamma$ , GM-CSF (granulocyte-macrophage colony-stimulating factor). At sub-stimulatory concentration, the initiating agents induce neutrophils physiological changes, but these cells do not release the contents of the azurophilic granules or stimulate the production of Oxygen radicals.

The initiated and activated neutrophils have significant functional differences. Due to secondary activating stimuli, they degranulate and release the intracellular enzyme increasing the intensity of respiratory metabolism tenfold, with the release of reactive oxygen species (ROS) [32,33]. Other functional properties of activated neutrophils are increased survival rate, the expression of endothelial selectins, afflux of neutrophils into the inflammatory site and synthesis and release rate of myeloperoxidase and elastase.

#### **2.4. Role of macrophages in the periodontal inflammatory-immune process**

Monocyte/macrophage is an essential cell in the immune response to pathogens: it triggers inflammation, activates the innate immune response, removes the dead tissues debris, and repairs tissue damage [34]. The activated macrophage is the bridge between innate and acquired immunity: it monitors the chemical quality of the microenvironment through surface receptors and through the secretion of cytokines and chemokines [28]. The inflammatory cells counts in the periodontal area, evaluated on histological sections of the human samples, has

the following distribution: B lymphocytes and plasma B cells (50-60%); Th and Tc lymphocytes (17%, with Th lymphocytes predominating over the Tc ones); macrophages and neutrophils (5-7%). Aggressive periodontal lesions and chronic forms have similar cellular populations [29]. Although in smaller proportion, macrophages play a very important role in innate and adaptive defense reactions through a large number of cytokines that they release: CSF (colony stimulating factors), ILs (interleukins), IFNs (interferons), TNF (tumor necrosis factor), growth factors and other chemokines [35]. *In vitro* analysis of macrophage gene expression profiles reveals that different Gram-negative and Gram-positive bacteria induce the transcription of a particular set of host genes, including those encoding proinflammatory cytokines [36].

Macrophages have a high functional plasticity, due to their localization in different tissues, but also in terms of their response to the diversity of signals received from the extracellular environment. i.e. depending on the specificity of the signals, they are functionally polarized. The macrophages polarization towards a pro-inflammatory (M1), anti-inflammatory (M2) or immune function regulatory status has been suggested by the distinct membrane markers profiles [32,37]. The two subpopulations M1 and M2, are mutually reversible. At different moments of the inflammatory process, macrophages pass from one state of polarization to another. Polarization is associated with chronic inflammation states, obesity, atherosclerosis, diabetes, allergy, asthma, autoimmune diseases, neoplasia [38]. In the periodontal inflammatory process, the macrophages are activated towards the M1 phenotype, following the initial pro-inflammatory reaction induced by bacterial LPS, pro-inflammatory ILs released by Th1CD4 + lymphocytes (IL-2, IFN $\gamma$ ), exhibiting paracrine and autocrine effects [39]. M1 macrophages control the acute phase of infection [40]. The M1 program consists of the activation of genes that encode pro-inflammatory ILs: IL-1, IL-6, IL-12, induction of nitric oxide synthase (iNOS), and in particular, TNF $\alpha$  synthesis, the main effector of tissue injury [41]. The M1 program protects against intracellular bacteria and allows the phagocytosis of tissue residues and of non-functional leukocytes, thus directly contributing to wound healing [42]. However, secreted TNF, proteases, IFN, peroxides release, angiogenic factors could have negative effects on the host tissues [43].

Functional polarization of M1 in periodontal lesions is demonstrated by the expression of specific membrane markers, but also by the high concentrations of TNF $\alpha$ , IFN $\gamma$ , and IL-2 in gingival biopsies, correlated with the severity of periodontitis (36). Excessive prolongation of M1 polarization can lead to tissue injury [44].

The repair of tissue damage begins only after the tissue and bacterial debris phagocytosis and corresponds to M2 polarization, which is activated by IL-4, IL-10, IL-13, TGF- $\beta$ , immune complexes, glucocorticoids [45]. A dual role is

assigned to IL-6, with stimulatory effects of both M1 and M2 activation programs, depending on the quantitative ratio of other ILs [46]. IL-4, released by Th2 lymphocytes, inhibits the inflammatory process and stimulates the M2 activation. M2 macrophages synthesize IL-4, IL-10, arginase, and TGF- $\beta$  with anti-inflammatory and immunomodulatory effects, and also proline found the chemical structure of collagen, having an role in the extracellular matrix production [47]. The M2 program, via the produces ILs, has therefore a pro-fibrotic effect and is associated with wound healing and tissue regeneration [47,48]. Unlike M1 macrophages which express HLA-DR and are antigen presenting cells, M2 macrophages do not present the antigens to Th lymphocytes and do not produce NO [49,50].

Some bacterial pathogens (e.g., the intracellular ones) specifically induce the activation of the M2 program and thus favor the chronic evolution of the infection [37].

Following the analysis of histological sections of healthy and periodontitis gingival tissue using the immunohistochemical method with specific monoclonal antibodies for the M1 and M2 markers, three macrophage subpopulations were identified: i) CD68 positive cells - M0 polarization; ii) CD68 and iNOS positive macrophages - M1 polarization (pro-inflammatory); iii) CD68 and CD206 positive macrophages - M2 polarization (tissue repairing) [51].

Their role in chronic gingivitis and periodontitis is not well established. M2 polarization was found in both gingivitis and periodontitis lesions, being related to the chronic inflammation, opposite to the acute phase, characterized by M1 polarization [37]. However, Benoit et al. reported that M2 polarization in gingivitis is similar to that found in the healthy tissue [52].

Gingivitis is characterized by a strong macrophages infiltration of the gingivae in comparison to healthy tissue [48]. In periodontitis lesions, HLA-DR, FGF (fibroblast growth factor) and TRAP (tartar-resistant acid phosphatase) expression is minimal, proving an impaired tissue immunosuppression [53]. On the other hand, infiltration of macrophages is to a great extent lesser in healthy gingivae, which suggests immunosuppression at the site of chronic inflammation, as a mechanisms that could limit the tissue destruction [54,55]. Some gingival sites are free of destructive periodontal disease and do not show functional M2 polarization [56].

### **3. The controversial role of *Staphylococcus aureus* in periodontitis**

*Staphylococcus aureus* is a common Gram positive aerobic bacterium but often behaves as an opportunistic pathogen and over the years have assumed the status of a `super bug` due its resistance to antibiotics, especially methicillin-resistant strains (MRSA), as well as the ability to form biofilms on medical

devices. The use of antibiotics in treating periodontal disease may lead to the increase of *Staphylococcus* spp. in the oral cavity. Consequently, the presence of a higher proportions of *S. aureus* in the oral cavity may lead to a significant increase in the incidence of periodontal infections and the difficulty of treating those that do occur [57]. Bacteriological, serological, anatomo-pathological results suggest that *S. aureus* plays a role in exacerbating dental diseases by forming a biofilm with the causative pathogens of periodontal diseases [58-62].

*S. aureus* is a predominantly extracellular infectious agent, but its protective mechanisms against phagosomal enzymes allow it to survive not only inside macrophages but also in the epithelial cells they invade [63]. In the gingival epithelial cells, *S. aureus* can survive for a long period, protected by the action of the immune effectors. However, in the early stage of gingivitis, *S. aureus* is not considered to be a key pathogen, just as the triad of 'red complex' bacteria (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) [64,65].

The reasons for implication of *S. aureus* as a periodontal pathogen could be explained by its inflammation-inducing structural components that are chemotactic for phagocytes, and induce the synthesis of chemo attractants, both *in vitro* and *in vivo*. These are phenol soluble peptides (phenol soluble modulins like peptides = PSM) that recruit neutrophils; lipoteichoic acid (LTA) and capsular polysaccharide induce IL-8 synthesis in circulating monocytes, epithelial and endothelial cells [66]; *S. aureus* activated endothelial cells synthesize IL-8 which stimulates neutrophil diapedesis. *S. aureus* virulence factors (TSST-1 - toxic shock toxin, enterotoxins A and B) induce IL-1 synthesis in monocytes [67-70]; TCD4 lymphocytes stimulated by the capsular polysaccharide synthesize chemokines that recruit neutrophils to the inflammatory site; PGN (peptidoglycan) induces C5a release (in C activation cascade), an intense chemotactic factor for neutrophils [71].

On the other hand, *S. aureus* has developed many strategies to avoid host immune effectors. It also has a vast repertoire of virulence factors [72], mostly with redundant activity. Furthermore, the bacterial cell is camouflaged with a protective capsular polysaccharide which inhibits *S. aureus* from phagocytes and antimicrobial polypeptides (AMP); *S. aureus* forms biofilms resistant to immune effectors; specific molecules that block the function of phagocyte receptors; toxins that induce the phagocytes lysis; CHIPS (chemotaxis inhibitory protein of *S. aureus*) blocks the receptor-mediated recognition of PAMPs, essential for the detection of bacterial invaders; other molecules secreted by *S. aureus* such as the C3-SCIN (staphylococcal C inhibitor) convertase blocker block the activation of the complement system, diminishing the phagocytosis of opsonized bacterial cells; iron capturing mechanisms; staphyloxanthin (carotenoid pigment) has a protective role against ROS action; proteases that hydrolyze all types of



antimicrobial proteins released from lysosomes [71]; toxins that induce the lysis of erythrocytes and leukocytes: hemolysin ( $\alpha$  toxin) and leucocidins [71,73]; superantigens (enterotoxins, toxic shock syndrome toxin - TSST and exfoliatin) with an immunosuppressive effect. Decreased efficacy of antimicrobial protective mechanisms explains the resistance of *S. aureus* to phagocytosis [31].

### **3.1. Exploitation of *S. aureus* dual pro-inflammatory / immune system evasion mechanisms for developing immunomodulatory therapies useful in the management of periodontitis**

Periodontitis and gingivitis are the two commonest diseases of the mankind. The aetiopathology of these diseases are classically based on microbiological and immune mediated theories [74]. Due to the failure in the current therapies based on a microbial etiology of periodontitis it is essential to explore new therapeutic pathways. Immunomodulation could possibly be one such avenue. The purpose of immunomodulation is the stimulation of non-specific immune reactivity as in the case of periodontitis or other infectious or neoplastic diseases, or alternatively, the inhibition of immune reactivity (e.g. organ transplantation, autoimmune diseases) [75].

Immunomodulators are biological or synthetic chemical compounds that interact with non-specific immune cells (macrophages, neutrophils), but also with an non-specific regulatory lymphocyte clones. These compounds exhibit either stimulatory or inhibitory activity, through cytokine-mediated signaling mechanisms. The fundamental principle of immunomodulation is the stimulation of the beneficial effects of phagocytes, but with diminishing their potentially harmful effects [76].

Most immunomodulators act indirectly by inducing cytokine synthesis in cells with non-specific immune function. In response to cytokine synthesis, lymphocytes alter their reactivity to different antigens. Bacterial lipopolysaccharides (LPS) are among the most critical immunomodulators, but their use is limited by the toxicity of the lipid component that causes hyperthermia, leukopenia, cardiovascular, and metabolic changes.

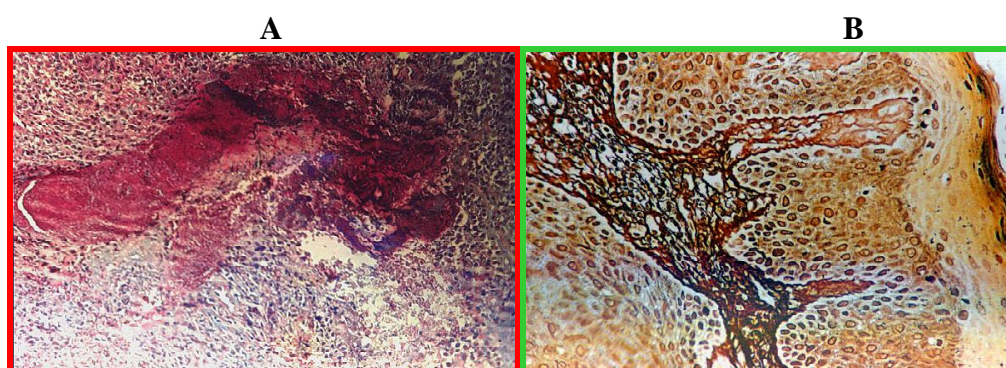
Due to these reasons, the National Development Institute for Microbiology and Immunology Cantacuzino, in Roamnia has over a period of 14 years (1997-2011) investigated the potential of a staphylococcal immunomodulator (IMS) in the management of periodontal disease. On this premise, the Institute has examoned the efficacy of a "Staphylococcal D Vaccine" Kit (consisting of four different dilutions, of one billion, 1/10, 1/100, 1/1000 of a bacterial mixture of 15 *S. aureus* strains/mL in physiological saline) [77]. Although the mechanism of action of IMS remains to be discovered, the clinical experience indicates that the `Staphylococcal vaccine` dramatically ameliorates periodontal inflammation, and significantly decreases the periodontal pocket depth [77,78]. The latter is due to

the secretion of collagenous tissue as well as the regeneration of the epithelium (Fig. 1).



**Fig. 1.** SAG 12 years - juvenile periodontitis - clinical signs of edema, and hypertrophy of the papillae, bleeding, reduced bacterial plaque, pockets of 7 - 8 mm in depth (A). After IMS local immunotherapy - reduction of inflammation, disappearance, normal-looking gum. Reducing the pockets depth (B).

Histological analysis of the gingival tissue reveals an intense repairing process, with the reorganization of the collagen fibers and the regeneration of the gingival epithelium (Fig. 2).



**Fig. 2.** Before treatment: microbial cells are observed, as well as cellular alterations of the chorion; HE staining x 64 (A). After treatment: detail, complete restoration of the collagenic axis of the interdenal papilla - Gomory HE staining x 100 (B).

#### 4. Discussion

The favorable effects of staphylococcal immunomodulator (IMS) was evident shortly after the treatment initiation. The anti-inflammatory effect of IMS was recorded in all patients with periodontitis, positive or negative for *S. aureus*, but without any underlying pathology (diabetes, AIDS, neoplasia).

It is tempting to hypothesize that the observed disease regression was due to the activated functionality of neutrophils and macrophages. We hypothesize that

the complex staphylococcal antigens mixture in IMS can induce the circulating neutrophil priming leading to diapedesis into the inflammatory gingival tissue. These cells in turn could then activate their enzymatic and respiratory potential ('respiratory burst'), described above, thus contributing to the elimination of inflammatory debris. Given the possible intracellular localization of *S. aureus*, the activation of a cellular specific immune response may also ensue. This was evidenced by the gingival epithelial cells infected with *S. aureus* which exhibited antigenic epitopes associated with both MHC I and MHC II molecules.

The local macrophages activated by IMS antigens secrete IL-1 that stimulates Th1 lymphocytes. Th-1 lymphocytes secrete a number of ILs that stimulate the specific cellular immunity, i.e.: IFN $\gamma$ , IL-2, and lymphotoxin  $\alpha$  (LT $\alpha$ ), which in turn are activating the TCD8 lymphocytes [48]. The TCD8 lymphocytes and a subpopulation of TCD4 lymphocytes with cytotoxic potential specifically recognize antigenic epitopes associated with MHC I and MHC II molecules [48]. IL-2 stimulates the proliferation of TCD8 and TCD4 lymphocytes, which target the modified epithelial cells, under the action of cytotoxic/ cytolytic factors or by expressing the Fas ligand. IL-2 inhibits B lymphocyte activation, that could explain the lack of a humoral response in periodontitis patients. The tissue repair could be attributed to the functional switch of M1 macrophages to M2, responsible of tissue repair and wound healing.

IMS antigens could also activate fibroblasts, which are essential for tissue repair, due to collagen synthesis.

## 5. Conclusion

Periodontal disease (BP) is an inflammatory condition of infectious nature that disrupts homeostasis of the periodontium, causing injury to the gums, alveolar bone, periodontal ligament, and cementum [10]. Different studies, with bacteriological, serological, histopathological data, suggest that *S. aureus* plays a decisive role in the progression of the pathological process of periodontitis towards osteitis and osteomyelitis [58,59].

Although the mechanism of action of IMS remains to be determined, the clinical experience indicates that the staphylococcal vaccine dramatically ameliorates periodontal inflammation and reduces the pockets depth, in particular by the secretion of collagenous tissue as well as the regeneration of the epithelium. In conclusion, IMS could be a useful adjunct in the management of chronic periodontitis although further work is required to substantiate our data.

## Acknowledgment

GRANT – VIASAN, Contract no. 333/2004 / - Romanian Academy, 2006.  
– Studies regarding the use of corpuscular staphylococcal vaccine in periodontitis treatment.

## REFERENCES

- [1] Ikeda E., Shiba T., Ikeda Y., Suda W., Nakasato A., Takeuchi Y., et al., 2019. Deep sequencing reveals specific bacterial signatures in the subgingival microbiota of healthy subjects. *Clin Oral Investig.*, 23(3), 1489–93.
- [2] Fragkioudakis I., Riggio M.P., Apatzidou D.A., 2021. Understanding the microbial components of periodontal diseases and periodontal treatment-induced microbiological shifts. *J Med Microbiol.*, 70(1).
- [3] Nyvad B., Takahashi N., 2020. Integrated hypothesis of dental caries and periodontal diseases. *J Oral Microbiol.*, 12(1), 1710953.
- [4] Chifiriuc M.C., Mihaescu G., Veronica L., 2011. *Medical Microbiology and Virology*. Bucharest: University of Bucharest; 747 pages.
- [5] Arweiler N.B., Netuschil L., 2016. The Oral Microbiota. *Adv Exp Med Biol.*, 902, 45–60.
- [6] Minty M., Canceil T., Serino M., Burcelin R., Tercé F., Blasco-Baque V., 2019. Oral microbiota-induced periodontitis: a new risk factor of metabolic diseases. *Rev Endocr Metab Disord.*, 20(4), 449–59.
- [7] Cavalla F., Biguetti C.C., Melchiades J.L., Tabanez A.P., Azevedo M. de C.S., Trombone A.P.F., et al., 2018. Genetic Association with Subgingival Bacterial Colonization in Chronic Periodontitis. *Genes (Basel)*, 9(6).
- [8] Boyer E., Martin B., Le Gall-David S., Fong S.B., Deugnier Y., Bonnaure-Mallet M., et al., 2020. Periodontal pathogens and clinical parameters in chronic periodontitis. *Mol Oral Microbiol.*, 35(1), 19–28.
- [9] Matsubara V.H., Bandara H.M.H.N., Ishikawa K.H., Mayer M.P.A., Samaranayake L.P., 2016. The role of probiotic bacteria in managing periodontal disease: a systematic review. *Expert Rev Anti Infect Ther.*, 14(7), 643–55.
- [10] Kinane D., Bouchard P., 2008. Periodontal diseases and health: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol.*, 35(8 Suppl), 333–7.
- [11] Duarte P.M., Bastos M.F., Fermiano D., Rabelo C.C., Perez-Chaparro P.J., Figueiredo L.C., et al., 2015. Do subjects with aggressive and chronic periodontitis exhibit a different cytokine/chemokine profile in the gingival crevicular fluid? A systematic review. *J Periodontal Res.*, 50(1), 18–27.
- [12] Ling M.R., Chapple I.L.C., Matthews J.B., 2015. Peripheral blood neutrophil cytokine hyper-reactivity in chronic periodontitis. *Innate Immun.*, 21(7), 714–25.
- [13] Hanke M.L., Kielian T., 2012. Deciphering mechanisms of staphylococcal biofilm evasion of host immunity. *Front Cell Infect Microbiol.*, 2, 62.
- [14] Scherr T.D., Hanke M.L., Huang O., James D.B.A., Horswill A.R., Bayles K.W., et al., 2015. *Staphylococcus aureus* Biofilms Induce Macrophage Dysfunction Through Leukocidin AB and Alpha-Toxin. *MBio.*, 6(4).
- [15] Gasner N.S., Schure R.S., 2012. Periodontal Disease. In *Treasure Island (FL)*.
- [16] Seminario-Amez M., López-López J., Estrugo-Devesa A., Ayuso-Montero R., Jané-Salas E., 2017. Probiotics and oral health: A systematic review. *Med Oral Patol Oral Cir Bucal.*, 22(3), e282–8.
- [17] Seeley J.J., Ghosh S., 2017. Molecular mechanisms of innate memory and tolerance to LPS. *J Leukoc Biol.*, 101(1), 107–19.
- [18] Bandara H.M.H.N., Panduwawala C.P., Samaranayake L.P., 2019. Biodiversity of the human oral microbiome in health and disease. *Oral Dis.*, 25(2), 363–71.
- [19] Murakami S., Mealey B.L., Mariotti A., Chapple I.L.C., 2018. Dental plaque-induced gingival conditions. *J Periodontol.*, 89 Suppl 1, S17–27.

- [20] Fine N., Chadwick J.W., Sun C., Parbhakar K.K., Khoury N., Barbour A., et al., 2021. Periodontal Inflammation Primes the Systemic Innate Immune Response. *J Dent Res.*, 100(3), 318–25.
- [21] Brown J.L., Johnston W., Delaney C., Rajendran R., Butcher J., Khan S., et al., 2019. Biofilm-stimulated epithelium modulates the inflammatory responses in co-cultured immune cells. *Sci Rep.*, 9(1), 15779.
- [22] Yucel-Lindberg T., Båge T., 2013. Inflammatory mediators in the pathogenesis of periodontitis. *Expert Rev Mol Med.*, 15, e7.
- [23] Benakanakere M., Kinane D.F., 2012. Innate cellular responses to the periodontal biofilm. *Front Oral Biol.*, 15, 41–55.
- [24] Meyle J., Chapple I., 2015. Molecular aspects of the pathogenesis of periodontitis. *Periodontol 2000*, 69(1), 7–17.
- [25] Meyle J., Dommisch H., Groeger S., Giacaman R.A., Costalonga M., Herzberg M., 2017. The innate host response in caries and periodontitis. *J Clin Periodontol.*, 44(12), 1215–25.
- [26] Wang Y., Andrukhov O., Rausch-Fan X., 2017. Oxidative Stress and Antioxidant System in Periodontitis. *Front Physiol.*, 8, 910.
- [27] Cekici A., Kantarci A., Hasturk H., Van Dyke T.E., 2017. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol 2000*, 64(1), 57–80.
- [28] Smith J.A., 1994. Neutrophils, host defense, and inflammation: a double-edged sword. *J Leukoc Biol.*, 56(6), 672–86.
- [29] Matthews J.B., Wright H.J., Roberts A., Cooper P.R., Chapple I.L.C., 2007. Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. *Clin Exp Immunol.*, 147(2), 255–64.
- [30] Scott D.A., Krauss J., 2012. Neutrophils in periodontal inflammation. *Front Oral Biol.*, 15, 56–83.
- [31] Sochalska M., Potempa J., 2017. Manipulation of Neutrophils by *Porphyromonas gingivalis* in the Development of Periodontitis. *Front Cell Infect Microbiol.*, 7, 197.
- [32] Shah R., Thomas R., Mehta D.S., 2017. Neutrophil priming: Implications in periodontal disease. *J Indian Soc Periodontol.*, 21(3), 180–5.
- [33] Miralda I., Vashishta A., Uriarte S.M., 2019. Neutrophil Interaction with Emerging Oral Pathogens: A Novel View of the Disease Paradigm. *Adv Exp Med Biol.*, 1197, 165–78.
- [34] DeLeon-Pennell K.Y., Iyer R.P., Ero O.K., Cates C.A., Flynn E.R., Cannon P.L., et al., 2017. Periodontal-induced chronic inflammation triggers macrophage secretion of Ccl12 to inhibit fibroblast-mediated cardiac wound healing. *JCI insight*, 2(18).
- [35] Zhang Y., Kuang W., Li D., Li Y., Feng Y., Lyu X., et al., 2021. Natural Killer-Like B Cells Secreting Interleukin-18 Induces a Proinflammatory Response in Periodontitis. *Front Immunol.*, 12, 641562.
- [36] Garaicoa-Pazmino C., Fretwurst T., Squarize C.H., Berglundh T., Giannobile W.V., Larsson L., et al., 2019. Characterization of macrophage polarization in periodontal disease. *J Clin Periodontol.*, 46(8), 830–9.
- [37] Guentsch A., Puklo M., Preshaw P.M., Glockmann E., Pfister W., Potempa J., et al., 2009. Neutrophils in chronic and aggressive periodontitis in interaction with *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. *J Periodontal Res.*, 44(3), 368–77.
- [38] Miller C.S., Ding X., Dawson D.R. 3rd, Ebersole J.L., 2021. Salivary biomarkers for discriminating periodontitis in the presence of diabetes. *J Clin Periodontol.*, 48(2), 216–25.
- [39] Zhang D., Xu T., Xu Q., Dong Q., Luo Y., Gao L., et al., 2021. Expression profile of macrophage migration inhibitory factor in periodontitis. *Arch Oral Biol.*, 122, 105003.

- [40] Fredriksson M.I., 2012. Effect of priming in subpopulations of peripheral neutrophils from patients with chronic periodontitis. *J Periodontol.*, 83(9), 1192–9.
- [41] Delves P.J., Roitt I.M., 1998. *Encyclopedia of immunology*. Academic Press.
- [42] Labonte A.C., Tosello-Tramont A.-C., Hahn Y.S., 2014. The role of macrophage polarization in infectious and inflammatory diseases. *Mol Cells.*, 37(4), 275–85.
- [43] Berglundh T., Donati M., 2005. Aspects of adaptive host response in periodontitis. *J Clin Periodontol.*, 32(s6), 87–107.
- [44] Nakao Y., Fukuda T., Zhang Q., Sanui T., Shinjo T., Kou X., et al., 2021. Exosomes from TNF- $\alpha$ -treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. *Acta Biomater.*, 122, 306–24.
- [45] Bertani F.R., Mozetic P., Fioramonti M., Iuliani M., Ribelli G., Pantano F., et al., 2017. Classification of M1/M2-polarized human macrophages by label-free hyperspectral reflectance confocal microscopy and multivariate analysis. *Sci Rep.*, 7(1), 8965.
- [46] Mills C.D., Ley K., 2014. M1 and M2 macrophages: the chicken and the egg of immunity. *J Innate Immun.*, 6(6), 716–26.
- [47] Sima C., Glogauer M., 2013. Macrophage subsets and osteoimmunology: tuning of the immunological recognition and effector systems that maintain alveolar bone. *Periodontol 2000*, 63(1), 80–101.
- [48] Mills C.D., Kincaid K., Alt J.M., Heilman M.J., Hill A.M., 2017. Pillars Article: M-1/M-2 Macrophages and the Th1/Th2 Paradigm. *J. Immunol.* 2000, 164, 6166-6173.
- [49] Holden J.A., Attard T.J., Loughton K.M., Mansell A., O'Brien-Simpson N.M., Reynolds E.C., 2014. *Porphyromonas gingivalis* lipopolysaccharide weakly activates M1 and M2 polarized mouse macrophages but induces inflammatory cytokines. *Infect Immun.*, 82(10), 4190–203.
- [50] Yang J., Zhu Y., Duan D., Wang P., Xin Y., Bai L., et al., 2018. Enhanced activity of macrophage M1/M2 phenotypes in periodontitis. *Arch Oral Biol.*, 96, 234–42.
- [51] Zhou L.-N., Bi C.-S., Gao L.-N., An Y., Chen F., Chen F.-M., 2019. Macrophage polarization in human gingival tissue in response to periodontal disease. *Oral Dis.*, (1), 265–73.
- [52] Benoit M., Desnues B., Mege J.-L., 2008. Macrophage polarization in bacterial infections. *J Immunol.*, 181(6), 3733–9.
- [53] Górska R., Gregorek H., Kowalski J., Laskus-Perendyk A., Syczewska M., Madaliński K., 2003. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. *J Clin Periodontol.*, 30(12), 1046–52.
- [54] Yu T., Zhao L., Huang X., Ma C., Wang Y., Zhang J., et al., 2016. Enhanced Activity of the Macrophage M1/M2 Phenotypes and Phenotypic Switch to M1 in Periodontal Infection. *J Periodontol.*, 87(9), 1092–102.
- [55] Martinez F.O., Gordon S., 2014. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep.*, 6, 13.
- [56] Orecchioni M., Ghosheh Y., Pramod A.B., Ley K., 2019. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol.*, 10, 1084.
- [57] Koukos G., Sakellari D., Arsenakis M., Tsalikis L., Slini T., Konstantinidis A., 2015. Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) in the oral cavity. *Arch Oral Biol.*, 60(9), 1410–5.

- [58] Cuesta A.I., Jewtuchowicz V., Brusca M.I., Nastri M.L., Rosa A.C., 2010. Prevalence of *Staphylococcus* spp and *Candida* spp in the oral cavity and periodontal pockets of periodontal disease patients. *Acta Odontol Latinoam.*, 23(1), 20–6.
- [59] Passariello C., Puttini M., Iebba V., Pera P., Gigola P., 2012. Influence of oral conditions on colonization by highly toxigenic *Staphylococcus aureus* strains. *Oral Dis.*, 18(4), 402–9.
- [60] Ansiliero R., Gelinski J.M.L.N., Samistraro Q.L., Baratto C.M., Almeida C.A., Locatelli C., 2021. Pathogenic Microbial Profile and Antibiotic Resistance Associated with Periodontitis. *Indian J Microbiol.*, 61(1), 55–65.
- [61] Chervinets V.M., Chervinets Y.V., Leont'eva A.V., Kozlova E.A., Stulov N.M., Belyaev V.S., et al., 2021. The microbiome of oral cavity patients with periodontitis, adhesive and biofilm forming properties. *Klin Lab Diagn.*, 66(1), 45–51.
- [62] Emery D.C., Cerajewska T.L., Seong J., Davies M., Paterson A., Allen-Birt S.J., et al., 2020. Comparison of Blood Bacterial Communities in Periodontal Health and Periodontal Disease. *Front Cell Infect Microbiol.*, 10, 577485.
- [63] Kim G.-Y., Lee C.H., 2015. Antimicrobial susceptibility and pathogenic genes of *Staphylococcus aureus* isolated from the oral cavity of patients with periodontitis. *J Periodontal Implant Sci.*, 45(6), 223–8.
- [64] Huang C.B., Alimova Y., Ebersole J.L., 2016. Macrophage polarization in response to oral commensals and pathogens. *Pathog Dis.*, 74(3).
- [65] Das A., Sinha M., Datta S., Abas M., Chaffee S., Sen C.K., et al., 2015. Monocyte and macrophage plasticity in tissue repair and regeneration. *Am J Pathol.*, 185(10), 2596–606.
- [66] Fritschi B.Z., Albert-Kiszely A., Persson G.R., 2008. *Staphylococcus aureus* and other bacteria in untreated periodontitis. *J Dent Res.*, 87(6), 589–93.
- [67] Chapple C.C., Srivastava M., Hunter N., 1998. Failure of macrophage activation in destructive periodontal disease. *J Pathol.*, 186(3), 281–6.
- [68] Lins R.D.A.U., Figueiredo C.R.L.V., Queiroz L.M.G., da Silveira E.J.D., Freitas R de A., 2008. Immunohistochemical evaluation of the inflammatory response in periodontal disease. *Braz Dent J.*, 19(1), 9–14.
- [69] Thorbert-Mros S., Larsson L., Berglundh T., 2015. Cellular composition of long-standing gingivitis and periodontitis lesions. *J Periodontal Res.*, 50(4), 535–43.
- [70] Chapple I.L.C., Mealey B.L., Van Dyke T.E., Bartold P.M., Dommisch H., Eickholz P., et al., 2018. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol.*, 89 Suppl 1, S74–84.
- [71] Lam O.L.T., McMillan A.S., Samaranayake L.P., Li L.S.W., McGrath C., 2013. Effect of oral hygiene interventions on opportunistic pathogens in patients after stroke. *Am J Infect Control.*, 41(2), 149–54.
- [72] Lam O.L.T., McGrath C., Bandara H.M.H.N., Li L.S.W., Samaranayake L.P., 2012. Oral health promotion interventions on oral reservoirs of *Staphylococcus aureus*: a systematic review. *Oral Dis.*, 18(3), 244–54.
- [73] Darveau R.P., Hajishengallis G., Curtis M.A., 2012. *Porphyromonas gingivalis* as a potential community activist for disease. *J Dent Res.*, 91(9), 816–20.
- [74] Kumar S., 2019. Evidence-Based Update on Diagnosis and Management of Gingivitis and Periodontitis. *Dent Clin North Am.*, 63(1), 69–81.

- [75] Papantonopoulos G., Delatola C., Takahashi K., Laine M.L., Loos B.G., 2019. Hidden noise in immunologic parameters might explain rapid progression in early-onset periodontitis. *PLoS One*, 14(11), e0224615.
- [76] Marciani D.J., 2017. Effects of immunomodulators on the response induced by vaccines against autoimmune diseases. *Autoimmunity*, 50(7), 393–402.
- [77] Stavaru C., Dumitrescu V., Lemeni D., Georgescu I.B.T., Codița I., Radu D.L., 2005. Microbiological and immunological study of staphylococcus vaccine effects in periodontitis. *Roum Arch Microbiol Immunol.*, 64(1–4), 11–6.
- [78] Georgescu T., 2004. Modulation of the immune response with staphylococcal vaccine in periodontal disease. University of Pitesti, editor. University of Pitesti Publishing House. Pitesti: University of Pitesti Publishing House, 290 p.