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REVIEW

The Immune Response in Periodontal Tissues

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Abstract The uniqueness of periodontal diseases is caused by several factors. This group of diseases is caused by numerous bacterial species formed in the dental biofilm, and one cannot distinguish the specific pathogen that is responsible for the disease initiation or progress (though Gram-negative anaerobic rods are associated with the advanced form of the disease). The disease is both infectious and inflammatory in its nature, and in the state of health there is always a subclinical level of inflammatory response, caused by the so-called harmless bacteria. Negligence in oral hygiene may result in maturation of the biofilm and trigger host response, manifesting clinically as gingivitis or—later and in susceptible subjects—as periodontitis. The article presents the contemporary knowledge of the inflammatory reaction occurring in tissues surrounding the tooth during periodontal inflammation. The most important mechanisms are described, together with implications for clinicists.

Keywords Periodontal diseases · Inflammation · Non-specific immunity · Mediators of inflammation

Introduction

The periodontal disease is an immunoinflammatory condition caused by a sophisticated polymicrobial structure, described as the dental biofilm. The contemporary concept of the pathomechanism occurring in periodontal inflammations regards it as a dual, infectious inflammatory process, consisting in two major pathogenic mechanisms (Aberg et al. 2015). The first one is direct toxicity of the microbiota-colonizing gingival sulcus, and in later stages of the disease, periodontal pockets. Enzymes and metabolites secreted by pathogens cause dysfunction or death of cells belonging to periodontal tissues, gingival epithelium, gingival connective tissue and periodontal ligament. That concept of periodontal tissue damage was the cornerstone of the specific plaque theory, which was developed in the 1970s (Theilade 1986). In the following years, scientists identified specific pathogen responsible for rare, rapid form of periodontal disease: *Aggregatibacter actinomycetemcomitans* was associated with aggressive periodontitis (whose localized form was previously described as juvenile periodontitis) (Slots 1976). Three anaerobic Gram-negative rods *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*, were found to be related with the severe course of chronic periodontitis (Socransky et al. 1998). Microbiological studies revealed that those species have unique features, some of which directly influence the immunological reaction of the host. *A. actinomycetemcomitans* presents on its surface a bacterial interleukin receptor, able to bind interleukin (IL)-1 (Dinarello et al. 2012). One of virulence factors secreted by *A. actinomycetemcomitans*, the cytolethal distending toxin, has been proven to enhance the expression of nuclear factor (NK)-κB ligand on host cells (the role of this ligand will be explained further). However, in vivo studies did not bring any proof that this phenomenon increases the progress of periodontal disease (Aberg et al. 2015). This proves that the disease process occurring in the tooth-supporting tissues is extremely complex and potential influencing factors, both bacteria- and host-associated have to be thoroughly studied. Such research had shown that the pathogenicity of *A. actinomycetemcomitans* and the progress of the periodontal

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disease are highly associated with secretion of leukotoxin A (Henderson et al. 2010). Leukotoxin A connects to the lymphocyte function-associated receptor 1 and disrupts the integrity of the target cell's membrane (Aberg et al. 2015). It also induces the massive proinflammatory response in macrophages (Kelk et al. 2003) due to strong activation of the inflammasome complex (Aberg et al. 2015). This mechanism may stand behind the strong association of *A. actinomycetemcomitans* species with aggressive periodontitis. *T. forsythia* secretes GroEL, which has been proven in vitro to synergize with IL-17 in bone resorption (Jung et al. 2016). *P. gingivalis* produces a wide variety of gingipains that are able to degrade critical elements of the immune response, such as complement components (C3 and C5), proinflammatory cytokines (e.g., IL-1, IL-6, IL-8) and immunoglobulins (IgA, IgM, IgG) (Barth et al. 2013). According to latest reports, *P. gingivalis* produces nucleases able to degrade neutrophil extracellular traps (NETs) (Doke et al. 2016).

Specific plaque theory was unable to explain the phenomenon of the same bacterial species identified both in healthy and diseased individuals (Papapanou 2002). Therefore, it evolved into the ecological plaque theory. In this theory, a shift of local conditions (particularly the change from supragingival to subgingival environment) favors specific pathogens, which leads from gingivitis to periodontitis (Marsh 1994). In the following years the concept of bacterial biofilm evolved. Due to that concept bacteria were not only considered to be immersed in the extracellular matrix. They were involved in the creation of a complex, three-dimensional structure, in which they were interacting with other species and significantly changing their phenotype (which could explain the presence of pathogens in healthy oral mouth) (Marsh 2005). Meanwhile, systemic factors modulating host response were identified (e.g., smoking and genetics) and found to be making the host "over-reactive" and prone to allow the progress of the disease (Genco 1996).

Bacteria-modulated immune response is the reason for damage to periodontal tissues. In this indirect mechanism, amplification of the immunological reaction results in destruction of periodontium and alveolar bone as a collateral damage (Van Dyke and Van Winkelhoff 2013). The non-specific (innate) component of the immune response is not aimed specifically at any antigen. Therefore, the immunological reaction is harmful not only to the microbial components of the dental biofilm, but also to the tissues supporting the tooth. Degradation of those results in clinically visible symptoms: bleeding gums, an increased depth of the periodontal pocket, an increased mobility of the tooth, and eventually, exfoliation. It is estimated that the secondary damage caused by the immunological reaction is responsible for approximately 80% of periodontal tissue damage (Grossi et al. 1994) (Fig. 1).

Therefore, the immunological reaction, especially its non-specific component, is the topic of great interest for all periodontists. In the beginning of the twenty-first century, modulation of host response was included in the treatment protocol of periodontal disease. Doxycycline in sub-antimicrobial doses is an established component of non-surgical treatment of periodontal disease in Western countries (Caton et al. 2000). The knowledge of the processes occurring during the immunological reaction is of utmost importance. The aim of this paper is to elucidate reactions taking place in host tissues after contact with an antigen, with special attention paid to the specificity of the periodontal area.

Periodontium and Periodontal Inflammation

The junctional epithelium and sulcular epithelium are unique forms of epithelial tissue, due to several reasons. They line the internal aspect of the gingival sulcus, the area, where the gums contact the teeth. Junctional epithelium contains epithelial attachment; above it is a space of the gingival sulcus, colonized by bacteria constituting dental biofilm. Below it is the periodontal ligament. Junctional epithelium has relatively wide intercellular spaces. Taking this into consideration, it is one of the thinnest membranes in the human body, separating external environment from internal environment (Shimono et al. 2003).

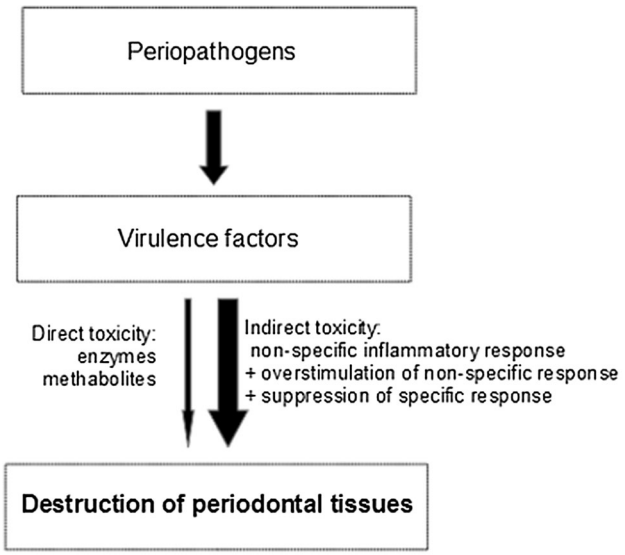

Fig. 1 Simplified diagram of bacterial toxicity in periodontium. The width of the *arrows* shows the strength of the action (acc. to Grossi et al. 1994)

Sulcular epithelium and the rest of oral mucosa contain dendritic cells (fixed macrophages), having the ability to present the antigen (Horewicz et al. 2013). The immune response, therefore, plays a crucial role in the disease process in periodontium.

The initial reaction of antigen's contact with host cells is a release of vasodilating agents. The ongoing inflammatory reaction causes an increase of permeability of endothelium of gingival blood vessels. The dynamics of the exchange of fluids between vascular and interstitial compartments is manifested by a change of tissue osmotic pressure (Lutfioglu et al. 2001). Clinical manifestation of this action is redness of gingiva, which in healthy state are pale pink, and the phenomenon of bleeding at the site after even gentle irritation, for example, during toothbrushing. There are several agents causing vascular relaxation. One of them is nitric oxide, secreted by macrophages infiltrating an inflamed tissue (Brennan et al. 2003) and gingival fibroblasts (Daghigh et al. 2002). An even more important vasodilating agent is histamine, secreted by mast cells (Wedemeyer and Galli 2000). Histamine is secreted in response to proinflammatory cytokines (predominantly IL-1 and IL-6) and complement protein fragments (predominantly C3a and C5a) (Krishnaswamy et al. 2001). Simultaneously, contraction of endothelial cells occurs, resulting in an increase of intercellular spaces and an increased permeability. Vasodilation causes a decrease of the bloodstream speed and a change of the flow pattern. White blood cells start to appear in the external part of the vessel lumen and contact with the vessel wall. The histamine-affected endothelial cells express E-selectin. This process is accelerated by IL-1 and tumor necrosis factor (TNF)-α. Leucocyte bonds to the selectin, then adheres more strongly via the integrins: vascular adhesion molecule and intercellular adhesion molecule (VCAM and ICAM, respectively). Integrins express on the surface of immunocompetent cells in response to the chemotactic agent. White blood cells migrate through the vessel wall and progress towards infection site (Timmerman et al. 2016). This is possible due to the chemotaxis phenomenon—the migration towards the increasing concentration gradient of a certain provoking agent (chemoattractant). This agent is usually the antigen, but IL-8, several component protein fragments and chemokines (inflammatory mediators produced by endothelial cells and leukocytes) can also increase leukocyte infiltration (Rossi and Zlotnik 2000). The first cells to dominate the inflammation site are neutrophils, then macrophages start to appear in greater numbers (after 24–48 h) (Ali et al. 2011). Both cell types are included in the non-specific inflammatory response, but their function is different.

Neutrophil Mechanisms

Neutrophils can release reagents into the surrounding environment (degranulation), or digest the pathogens (phagocytosis). Degranulation occurs when active substances contained in specific cytoplasmic organella (granules) are released out of the cell. In this pattern, mast cells release histamine. The impulse for degranulation is usually the antigen bound with a specific antibody. When that complex anchors the receptor for the Fc fragment, degranulation occurs. Substances contained in granules are mostly enzymes. Myeloperoxidase causes synthesis of hypochlorous acid, which is highly toxic to the surrounding cells (both bacterial and host) (Alfakry et al. 2016). Neutrophil elastase is known to destroy membrane proteins of several bacterial species, but is also responsible for degradation of type IV collagen and elastin in gingival connective tissue and periodontal gap (in which periodontal ligament links the tooth cementum with the alveolar bone) (Alfakry et al. 2016). Cathepsins family is a group of cysteine proteases. Although they are usually activated in low pH (like in lysosomes), they may also function in an extracellular environment, such as inflammation site which usually has acidic pH. Beside that cathepsin K, one of the most potent collagen-degrading enzymes, is active extracellularly. Beside neutrophils, it is also secreted by osteoclasts and plays a key role in the destruction of the bone tissue (Wen et al. 2016). Another group of enzymes responsible for the degradation of connective tissue is the group of proteolytic enzymes catalyzed by the zinc ion. Because of this property they are named matrix metalloproteinases (MMPs). They include collagenases, gelatinases, stromelysins, matrilysins and several others. From the periodontologist's point of view, the most important are collagenases and gelatinases, capable of degrading type IV collagen, predominant in periodontal tissues (Alfakry et al. 2016). Lysozyme is present not only in white blood cells, but also abundantly in saliva. It is responsible for destruction of peptidoglycans. They are mostly present in the bacterial wall; therefore, lysozyme is especially effective in defense against Gram-positive bacteria (Surna et al. 2009). Another antibacterial agent present both in saliva and neutrophil granules is lactoferrin, specific transporting protein that binds iron, essential for bacterial growth. It binds also to the bacterial wall and greatly increases its permeability, which leads to breakdown of the targeted cell (Glimvall et al. 2012). Since both lysozyme and lactoferrin are present not only in neutrophil granules, but also in saliva and milk, their role is much greater in very early stages of human life (Surna et al. 2009). Another protein, which interacts with the bacterial membrane, is the bactericidal permeability increasing

protein. It connects to lipopolysaccharides, which are a component of the Gram-negative bacteria membrane (Glas et al. 2006).

The second feature of neutrophils is phagocytosis. Whenever that is not the first contact with a certain antigen (the most frequent condition), there are antibodies present in the periodontal tissue that interact with the pathogen as soon as it appears. Antibodies have coat (opsonize) the antigen, enhancing the process of phagocytosis. Neutrophils ingest the pathogen, enveloping it in a specific vesicle (phagosome); NADPH oxidase bound to the membrane of the phagosome activates during the engulfment of the pathogen, and releases an electron from the intracellular NADPH. The electron is collected by oxygen, which results in creation of a superoxide anion that kills the pathogen. The excessive process of the so-called respiratory burst leading to the release of the reactive oxygen species, may lead to destruction of cells of periodontal tissues through disruption of the nuclear DNA or cytoplasmic proteins and enzymes, as well as serving as potent chemotactic agents (Ozmeric 2004).

A recently discovered function of neutrophils is the ability to create NETs (Vitkov et al. 2009). Those are structures formed from nuclear chromatin bound with a wide spectrum of antibacterial compounds (Brinkmann et al. 2004). NETs are able to neutralize the virulence factors or to eliminate the periopathogens (Brinkmann et al. 2004). NETs are either produced by neutrophils or created after their reactive oxygen species-dependent cell death (NETosis) (Li et al. 2010). NETs can be created as a response to bacteria or their components (lipopolysaccharides), fungi, or to the signal from proinflammatory cytokines (Doke et al. 2016). Described neutrophil functions in the periodontal tissues have been presented in Fig. 2.

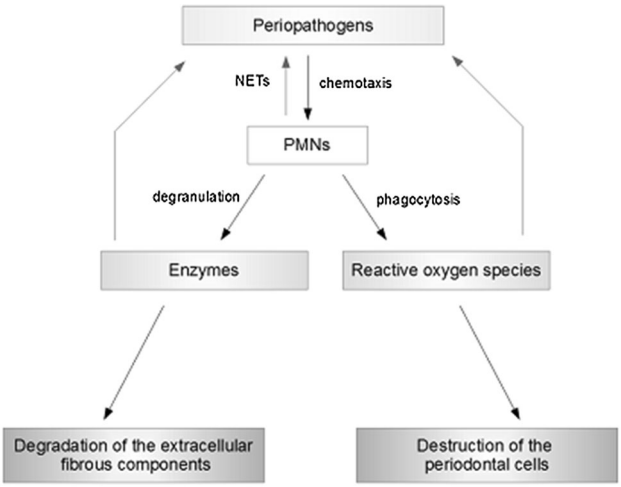

Fig. 2 Interaction between periopathogens and neutrophils (PMNs). Antigen binding causes degranulation and phagocytosis, which—together with neutrophil extracellular traps (NETs)—neutralize the bacteria (*gray arrows*), but—as a collateral damage—destroy periodontal tissue (*black arrows*)

Macrophage Immunity

Other cells greatly involved in the inflammatory infiltration of initial stages of periodontal disease are macrophages. Although their name partially describes their function, they also play a role in amplification of the inflammatory reaction, as well as in lymphocyte recruitment. Phagocytosis, however, remains their main function. They usually appear in larger numbers at the inflammation site after the first response triggered by neutrophils, which usually lasts 48 h and is histologically described as the initial stage of gingivitis. Macrophages remove aged neutrophils expressing CD31 on their surface and dead cells, both host and alien. The process of phagocytosis was described earlier: removed cells are engulfed and ingested, forming a phagosome which further connects with a lysosome. The reactive agents released during this connection cause breaking down of the substances in the phagolysosome.

Although macrophages were mentioned earlier as potential amplifiers of the inflammatory process, more accurate description would be regulators of the host response.

Macrophages' regulatory function in periodontium comes from the fact that they supervise two major remodeling processes occurring in the periodontal tissues. One of them occurs in the connective tissue and depends on the equilibrium between MMPs and their tissue inhibitors (TIMPs). The second occurs in the alveolar bone and depends on the balance between osteoblasts and osteoclasts. MMPs, as mentioned earlier, are secreted by the neutrophils migrating into an inflamed gingival or periodontal pocket. TIMPs are relatively small proteins present in the tissue and capable of binding to MMPs, thus neutralizing its proteolytic activity. It is believed that the imbalance between MMPs and TIMPs may lead to extensive degradation of the extracellular matrix on one the hand, or to fibrosis on the other hand (Hannas et al. 2007). Alveolar bone tissue metabolism relies on the equilibrium between the formation and resorption. This depends on whether the osteoblastic or osteoclastic activity will play the primary role. To elucidate the mechanism behind maintaining this balance, NF-κB must be described. This abbreviation stands for NK-κ light-chain enhancer of activated B cells. It is an intracellular protein responsible for triggering cellular response to external stimulus, via kinase activation (Baldwin 1996). Its receptor activator (RANK) is the triggering point of the osteoclast formation and activation. This starts with binding of RANK ligand

(RANKL), which in the state of disease may be synthesized by Th1, Th17 or B cells. Binding is prevented if RANKL is bound with osteoprotegerin, expressed by mesenchymal stem cells. This mechanism seems to play an important role in the clinical differentiation of the aggressive and chronic forms of periodontal disease, as well as between reversible (gingivitis) and irreversible (periodontitis) inflammatory process (Garlet 2010).

The key role of macrophages in the immune response in periodontal tissues comes not only from the fact of partial or full participation in catabolism of the connective tissue and bone. Another function of macrophages is acting as a trigger for both innate and adaptive immune response. Macrophages and dendritic cells (there is an ongoing discussion whether those two cells come from the same trait or not) (Ginhoux et al. 2016) express specific type of receptors on their surface. Toll-like receptors (TLR) are capable of recognizing the pathogen as soon as it passes through the mucosal barrier. The binding between a TLR and an antigen activates the host cell to secrete proinflammatory mediators and initiate the whole aforementioned sequence of the non-specific inflammatory response (Garlet 2010).

That pathogen response is mediated via an intracellular signaling pathway. The recently discovered and described inflammasome is a protein complex in cytoplasm responsible for activation of the proinflammatory cascade. It is playing a key role in the maturation of proinflammatory cytokines, such as IL-1 and IL-18, from their inactive (pro-IL-1) to reactive form. Therefore, inflammasome is also of major importance for the regulation of the innate immune response.

Bacterial antigens binding to the pattern recognition receptors (e.g., TLRs) activate intracellular nucleotide-binding-domain-like receptor (NLR). This activates biologically neutral pro-interleukin to interleukin. Simultaneously, a second activation signal is required to activate pro-caspase-1 into caspase-1. It is provided by activation of the danger recognition receptor (DAMP), such as extracellular ATP or cholesterol crystals (Yilmaz and Lee 2015). The common source of the extracellular ATP is the host cells—dead or subjected to infection or stress (Fig. 3). It is considered to be one of the most potent proinflammatory stimuli (Gombault et al. 2013), and therefore may play a crucial role in the regulation of host response to periopathogens. It is even considered to be the master regulator of the ATP-induced IL-1 secretion (Yilmaz and Lee 2015). Its receptor, P2X7, is commonly present in gingival epithelial cells (Yilmaz and Lee 2015).

The disregulation of inflammasome function may be one of the major factors influencing the progress and severity of the periodontal disease, as the innate immune response, and in particular, proinflammatory cytokines have a great influence on destruction of tooth-supporting tissues (Bostanci et al. 2009). NLRP3 inflammasome is considered to be the most important, and therefore, most thoroughly studied so far due to its potential to bind numerous bacterial antigens (Latz 2010). Several periopathogens are targeting the inflammasome with their virulence factors. In that way, microbial activity may modulate and disrupt the inflammatory response (Taxman et al. 2010). The P2X7 receptor binding extracellular ATP has been shown to be disrupted by the *P. gingivalis* species (Choi et al. 2013).

Macrophages and dendritic cells possess also the property of presentation of the antigen, which makes them the keystone between innate and adaptive inflammatory reaction. An antigen is presented to the $CD4^+$ cells (T helper lymphocytes), which will lead to production of specific antibodies. In case of future contact with the same antigen, antibodies opsonize it making the phagocytosis much more effective. Innate immune response, due to its specificity, is directed against pathogens only. One can distinguish its cellular and humoral component, though if a specific inflammatory reaction is triggered (and that happens always during the second and further contacts with the antigen), both components are always active. The specific cellular response involves several types of T cells. Those are Th cells, responsible for initiation of the specific response, T cytotoxic cells, involved in the specific phagocytosis, and T suppressor cells, quenching host defense reactions. Th cells, after contact with a macrophage presenting an antigen, can stimulate other components of the immune response, both specific (T cytotoxic cells) and non-specific (neutrophils and macrophages). They can also transform B cells into plasma cells which dominate in the stabilized periodontal defect (Berglundh and Donati 2005). Th cells can be sub-divided into populations of cells with slightly different properties. Th1 lymphocytes take a greater part in the cellular component of the adaptive immune reaction; Th2 cells primarily affect B cells and the humoral response. The fact that Th2 cells are connected with the aforementioned maturation of plasma cells, together with the fact of infiltration of plasma cells in the advanced periodontal defect, probably leads to the assumption that Th2 cells are associated with progression of periodontal disease (Gemmell et al. 2007). Th17 lymphocytes secrete cytokines of proinflammatory properties, Treg cells, formerly described as Ts lymphocytes, suppress the inflammatory response both in general as well as regarding the specific antigen (over-reaction) (Gaffen and Hajishengallis 2008). That role is partially attributed to the cytotoxic T lymphocyte-associated molecule 4 expressed by Tregs. It is expressed in higher numbers in patients suffering from periodontitis and seems to inhibit the specific cellular immune response. Transforming growth factor-β secreted by Treg cells slows periodontal disease progression. It suppresses secretion of

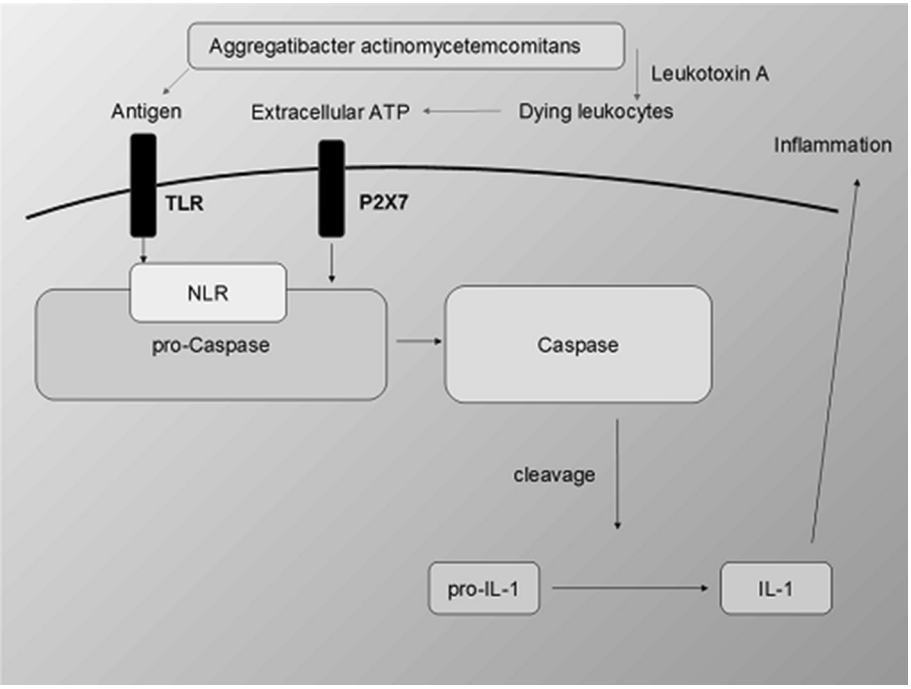

Fig. 3 A cascade of reactions occurring in the inflammasome complex, together with the hypothetic mechanism of aggressiveness of *A. actinomycetemcomitans* species, leading to aggressive periodontitis. Transforming pro-caspase into caspase able to cleavage pro-IL-1 into active IL-1 requires two parallel signals. The first one comes from the activation of nucleotide-binding-domain-like receptor (NLR) by the toll-like receptor (TLR) which connects the antigen. The second signal is the result of P2X7 receptor binding the extracellular ATP (modified after Aberg et al. 2015)

numerous proinflammatory cytokines as well as endogenous MMPs (Cardoso et al. 2008). T cytotoxic cells are responsible for phagocytosis of the cells expressing non-self proteins in major histocompatibility complex type I molecules. Therefore, they participate in the response against viral infections, but also in elimination of neoplastic or alien cells (as in transplanted organs), and do not have great influence on the immunological reaction taking part in periodontal tissues (Berglundh and Donati 2005).

Humoral Component

B cells, together with their periodontal tissue form (plasma cells), take a great part in the immunological response in periodontal disease (Berglundh and Donati 2005). They produce specific antibodies directed against periodontal pathogens. They secrete a spectrum of inflammatory mediators, like IL-1 or TNF-α, acting synergistically. Finally, they possess the property of presenting the antigen, thus further stimulating the inflammatory reaction. In the course of periodontal disease, pathogens infecting host tissues stimulate plasma cells to secrete RANKL, described before as the stimulator of osteoclastogenesis (Kawai et al. 2006).

Antibodies released by plasma cells have several functions. They coat the pathogen (opsonize) enabling easier phagocytosis by macrophages, or neutralize the specific function of the antigen by simple occupation of the active region of the pathogen's receptor (Xynogala et al. 2009). Plasma cells in periodontium produce mainly class G immunoglobulins (IgG), and lower numbers of class A immunoglobulins (IgA) (Ebersole 2003). On the other hand, saliva is a vast source of IgA, and possesses the ability to neutralize several bacteria responsible for the progress of periodontitis (Jorgensen et al. 2010). In contrast to the previously mentioned thesis of Th2 cell-mediated severity of periodontal disease, there are studies that suggest that the strength of the humoral component of the immune response is an indicator of maintaining periodontal health, or positive resolution of periodontal disease. Serum levels of the IgG antibodies directed against specific periopathogens are higher in individuals with a better state of periodontal tissues (Rams et al. 2006). The difficulty in establishing the nature of the immune response and its influence on the clinical symptoms of periodontal healing comes from the fact that, unlike in most systemic infections, periodontal disease is caused by a vast variety of pathogens, forming specific structure (dental biofilm) capable of withstanding high concentrations of antimicrobial agents and altering properties of the bacterial species involved (Kouidhi et al. 2015). It is crucial to realize the fact that in periodontal disease factors that act as multipliers of the immunological reaction also destroy periodontal pathogens (Garlet 2010). So only monitoring the periodontal disease as a whole may give an answer to the question, if the specific pathway of the immune reaction is beneficial or not for tissues surrounding the tooth. In vitro studies, carried out in highly controlled conditions, are useful in discovering the nature of the specific particle,

protein or cellular process, but conclusions drawn from their results cannot be directly matched to the in vivo situation.

Practical Aspects

The knowledge of ongoing immunological processes has been utilized both in diagnostics and treatment. Obviously, every aforementioned aspect of the immune response: cell function, mediator or receptor, has been studied by researchers to establish its role in the clinical manifestation of the disease process (Ali et al. 2011; Ozmeric 2004). Inflammatory mediators, being signal carriers between different components of the immunological response, are the subject of special attention. Those studies are conducted to elucidate the complexity of the host defense reaction, and also in hope of discovering the single keystone mediator that would be a good diagnostic or prognostic marker. Besides the difficulties coming from the tangled immune processes, there was also a problem of the medium in which the marker should be diagnosed. Tissue biopsies could not be utilized, since the sampling would be too invasive for patients in comparison to the damage coming from the disease process (Lomba et al. 2015). For the same reasons blood examination would be problematic (Shetty et al. 2013). GCF examination required sophisticated and expensive equipment (Perozini et al. 2010). In the mid of 1990s, one could think the perfect answer to those problems was given. Two of the single nucleotide polymorphisms, both located in the IL-1 gene cluster, seemed to strongly correlate with loss of periodontal attachment in non-smoking adult individuals. Individuals with mutated IL-1 gene developed stronger inflammatory response to the same bacterial load (Kornman et al. 1997). This was the basis for development of the first commercially available genetic test utilized in periodontology. It was supposed to efficiently predict the course and tempo of periodontal inflammation. Further studies were bringing ambiguous results, and until now it is yet not clear whether one can prognose future development of the periodontal disease process basing on mutation of the IL-1 gene. It seems likely that predisposition to the disease and its outcome may be, however, at least partially, genetically determined (Karimbux et al. 2012).

Another attempt to utilize the knowledge of immunological processes occurring in the periodontium was made in 2008 (Offenbacher et al. 2008). Previously developed periodontal disease classifications had focused on distinguishing the disease forms basing on clinical symptoms. Offenbacher et al. (2008) tried a different approach. He monitored titers of IgG's against most known periodontal pathogens in serum. Then he grouped the patients according to the increasing levels of serum antibodies. He found that he could correlate the clinical state with the antibodies' levels, and that actually only two periodontal parameters are needed to effectively group the patients (Offenbacher et al. 2008). This scale, although simple, has failed so far to be introduced in general practice.

Effective and established utilization of the knowledge of immunological processes occurring in the periodontium is found in modern treatment protocols. Awareness of the importance of non-specific inflammatory response in periodontal tissue destruction caused an introduction of inflammatory modulators. At the beginning, topical and systemic non-steroid anti-inflammatory drugs were proposed. The next generation of drugs was lipoxins or resolvins (endogenous or exogenous lipid inflammatory suppressors). Numerous studies show the efficacy of resolvins in suppression of neutrophil infiltration, increased removal of apoptotic neutrophils from the inflammation site without secreting proinflammatory cytokines, and amplification of periodontal regeneration processes (Schwab et al. 2007).

In the Western countries, a popular adjunctive therapy (to standard periodontal treatment) is the use of sub-antimicrobial dose doxycycline. This medicament (20 mg tablets of doxycycline, in comparison to 100 mg standard bacteriostatic dose) is aimed at inhibiting neutrophil collagenase. Clinical studies show that treatment with low-level-doxycycline effectively improves the periodontal state, when compared to the classical therapy (Sgolastra et al. 2011).

Conclusions

Immunological response in periodontal tissues is a highly complex process. An additional difficulty in understanding its mechanism is the multitude of potential pathogens. Meyle and Chapple (2015) in their paper elegantly summarized the current state of the knowledge. In the periodontal area, there is a constant interaction between bacterial biofilm and host defense system. In the state of health there is a subclinical mobilization of immunocompetent cells, as pioneer bacteria are weak provokers of host response. If biofilm is not removed (in case of poor oral hygiene), the so-called "quorum sensing" pathogens appear, enable of initiating exaggerated immune response. Proinflammatory mediators in susceptible patients cause further destruction of the connective tissue and bone, as the protective action of TIMPs and antioxidants is insufficient to stop the amplified inflammatory outbreak (Meyle and Chapple 2015). The advances in immunological science give a hope of identifying the main factors responsible for the disease progress and their efficient elimination. The

classic periodontal treatment protocols were aimed at reducing the infective component and surgical correction of the damage occurring in hard and soft tissues. The novel approach, bringing more attention to the modification of host response and reducing the "over-reactive" element, may bring a hope of cost-effective non-surgical, successful periodontal therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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