Review paper

The immunologic aspects of periodontal disease

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Abstract

This article summarizes some experimental and clinical data about immunologic determinants of etiopathogenesis of periodontitis. The main etiological factor of periodontitis is bacterial plaque deposited on the tooth surface. In the healthy oral cavity there is a state of balance between bacterial antigens and host immune system. The pathogenic bacteria interact with periodontal tissue, stimulating local immune reaction within the oral cavity. Chronic periodontitis is the most prevalent periodontal disease in adults. The immunologic inflammatory reaction, leading to periodontal tissue destruction, is initiated by Gram-negative bacteria. Oral mucosa is infiltrated by immune cells, which take part in cellular immune reactions and humoral response. The research directions in the field of immunologic aspects of periodontitis, pointed out in this paper, clearly indicate the complexity of the pathological processes, leading to destruction of periodontal tissue.

Key words: *periodontitis, etiology, bacterial antigens, host response, immune system.*

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The pathogenesis of periodontal disease is multifactorial and based upon a complex interaction of genetic, environmental, and immunological factors. The substantial defect of the mucosal barrier, dysregulation of immune response, and chronic inflammation of the mucosa are causing the chronic pathological process. The main etiological factor of periodontitis is bacterial plaque deposited on the tooth surface. In the healthy oral cavity there is a state of balance between bacterial antigens and host immune system; once it gets disturbed, periodontal tissue is subjected to irreversible damage. Low oxidation-reduction potential as well as oxygen partial pressure below the gumline favors colonization of this area mainly by anaerobic bacteria species. The invasion of periopathogenic microorganisms leads to widening of capillaries and increased endothelium permeability. Adhesive molecules, called addressins, appear on the surface of endothelial cells; they stimulate the leukocyte migration by binding to the receptors expressed on their surface. In the next phase an inflitration forms below the junctional epithelium of gingival crevice. The plasmocytes, neutrophils, and macrophages pass into the gingival crevicular fluid. Upon stimulation by bacterial antigens and toxins, the inflammatory cells secrete mediators of inflammation, i.e. cytokines and prostaglandines that ultimately lead to activation of immune cells. Proteolytic enzymes are also secreted by these cells. Together with fagocytosis, it leads to the ongoing destruction of periodontal tissue [1-3]. Chronic periodontitis is the most prevalent periodontal disease in adults. The immunologic inflammatory reaction, leading to periodontal tissue destruction, is initiated by Gram-negative bacteria. The main periopathogenic bacteria isolated from periodontal pockets in course of chronic periodontitis are: *Porphyromonas gingivalis*, *Tannerella forsythia* (*Bacteroides forsythus*), *Treponema denticola*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens*, *Campylobacter rectus*, *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, *Streptococcus intermedius*, *Peptostreptococcus micros*, *Capnocytophaga sputigena*, *Capnocytophaga ochracea* [4, 5]. The pathogenic bacteria interact with periodontal tissue, stimulating local immune reaction within the oral cavity. This reaction mainly comprises of two basic mechanisms leading to the elimination of antigen: secretion of specific antibodies and development of adaptive immunity. Immunoregulation is a conception of complex process that determines the orientation, intensity, rate, and duration of immune response. Immunoregulation depends on mechanisms, in which specific antibodies, effector cells of immune system, accesso-

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ry cells, and humoral mediators take part. Thus the intensification or inhibition of immune response may be realised by the conventional phenomenon of biofeedback. It plays a role in the regulation of production of antibodies as well as anti-idiotypic antibodies, secretion of humoral mediators by the stimulated T cells, interaction between the macrophages and B cells or T cells, as well as the direct interaction between T-T cells or T-B cells. Oral mucosa is infiltrated by immune cells. Some of them, such as polymorphonuclear lymphocytes, monocytes, B cells, and T cells take part in cellular immune reactions, while IgG, IgA, IgM are the active component of humoral response. On the other hand, sIgA, IgG, mucins, PRP3, histatines, and defensins are mediators of humoral response of salivary origin. It should be noticed that upon induction of antigen specific immune reaction the composition of gingival crevicular fluid changes significantly. Many authors believe that the assessment of antibodies in the gingival crevicular fluid in course of periodontitits can serve as an indicator of local humoral response. Moreover the peripheral IgG against specific RgpA-Kgp complexes of *Porphyromonas gingivalis* has been proven to be significantly upregulated in sera of the patients with chronic periodontitis, as compared to the clinically healthy subjects [6]. Ebersole *et al*. have observed increased IgG level in the inflammatory infiltration of periodontal pocket as a response to the bacterial pathogens [7]. The authors of the study have proven a correlation between IgG concentration in the gingival crevicular fluid and in the peripheral blood. Thus it may be concluded that increased IgG level corresponds to the ongoing inflammatory process. On the other hand, peripheral IgG level has been found to be significantly higher in both aggressive and chronic periodontitis than in healthy control group [8]. Moreover a positive correlation between IgA and IgG level in saliva and peripheral blood serum was observed in periodontitis [9]. An effective host defence requires specific immune response, which is referred directly against the periopathogens. The main group of cells, that are genetically programmed to recognize and fight the infective microorganisms, are antigen presenting cells, B cells, and regulatory T cells (cytotoxic Tc cells, helper Th cells, suppressor Ts cells, and contrasuppressor Tcs cells). Many authors have investigated the CD4+ T cells, also referred to as T helper cells. There are at least two distinct CD4+ cell populations: Th1 and Th2 cells. According to some studies on the Th1 and Th2 cells, as well as their secretion profile in course of periodontal diseases, their role is of immunoregulatory character [10]. The stimulation of either Th1 or Th2 cell population depends on the character of infective agent. Each of them determines the induction of specific effector mechanisms. Th1 cells secrete IL-2 and IFN-γ, leading to CD8+ cytotoxic cells proliferation, autocrine Th1 response stimulation, increase of the macrophages activity and inhibition of Th2 cells functions. On the other hand, the expression of Th2 cells and cytokines produced by these cells (namely: IL-4 and IL-10) lead to IgG1 and IgE synthesis, Th1 inhibition, and monokines production. Apparently it's the antigen properties that determine the immune response and progress of specific Th cytokines profile. Th1 cells are believed to be directed mainly against the intracellular pathogens, while the extracellular mikcroorganisms are destroyed by the humoral reaction [11]. Bártová *et al*. have investigated the cytokine profile of activated Th2 cells in patients suffering from aggressive periodontitis. They have observed a significantly increased IL-4 level as well as decreased IFN-γ level in aggressive periodontitis as compared to the healthy control [12]. These observations were not supported by other authors. Assessing the IFN-γ and IL-4 level that corresponds to the T cell subpopulation activity, they have not found significant difference between aggressive periodontitis and control group. Neither the peripheral level of CD3+, CD4+, and CD8+ T cells, nor the proportion of CD4+/CD8+ cells differed in patients as compared to the control. Moreover no significant changes were observed for CD45RA naive T cells (mature T cells that have not encountered their cognate antigens within the periphery) to CD45RO memory T cells proportion [13]. The first phenomenon within adaptive immune response pathway is presenting antigen to the B cells. Each antigen presenting cell (APC) has B7 molecules on its surface. They can be divided into B7-1 and B7-2 (or CD80 and CD86, according to the current terminology) group. They bind to the CD28 or CTLA4 antigens on T helper cells. The physical interaction between antigenic specific B and T cells is simplified thanks to the ligand-receptor interaction that is responsible for signaling between these cells. For example the differentiation antigen CD40, belonging to the TNF (tumor necrosis factor) family and present on B cells, binds to the CD40L antigen on T cells. The stimulated T cells secrete range of cytokines, activating the proliferation and differentiation of immune effector cells, such as IL-10 or TGF-β (transforming growth factor β). Aoyagi *et al.* evaluated the specific reaction taking place in chronic periodontitis and in healthy control group upon stimulation with cellular membrane antigens of *Porphyromonas gingivalis*. They have assessed the level of differentiation antigens CD28 and CTLA4, ligand for CD40 (CD40L) and the expression of IL-10 and TGF-β mRNA [14]. According to this study the expression of IL-10 mRNA was significantly increased in the course of periodontitis, while the TGF-β mRNA expression did not show any alterations. Moreover the CTLA4 molecule was the only diferentiation antigen exhibiting significant up-regulation in periodontitis patients as compared to the control group. Based on these findings, the stimulation by bacterial antigens does not activate cellular response, still the T cell functions can be regulated by the expression of CTLA4 ligand that may play an important role in the etiopathogenesis of periodontal disease. One of the extensively studied areas is the cytokines of bone

turnover OPG/RANKL. The pathogenic bacteria have been found to induce osteopoetine and RANKL synthesis within periodontal tissue. Both of them influence osteoclasts differentiation. Osteopoetine is responsible for osteoclasts activation during bone resorption, while RANKL is a RANK (receptor activator of nuclear factor κB) ligand. Binding of RANK and its ligand RANKL is a prerequisite for osteoclasts differentiation and bone tissue destruction. Osteoprotegerin (OPG, also known as osteoclast binding factor, OBF, or osteoclast inhibitory factor, OCIF), on the other hand, is inhibitor of the osteoclasts [15]. Osteoprotegerin competes with the RANK receptors presented on the cell surface of immature osteoclasts, binding to the receptor activator of nuclear factor κB ligand (RANKL, also called osteoclast differentiation factor, ODF), and thus preventing bone resorption. Osteoprotegerin is closely related to TNF type II receptor, as well as differentiation receptor CD40 [16]. According to the studies performed by Teng *et al*., blocking of RANKL by the OPG can suppress destruction of alveolar bone and osteoclasts proliferation. Moreover they have proven the induction of RANKL synthesis by CD4+ T cells receptors stimulation by *Aggregatibacter actinomycetemcomitans*, with RANKL being a key mediator of osteoclastogenesis. Kikuchi *et al.* have proven *in vitro* that the LPS isolated from *Aggregatibacter actinomycetemcomitans* can stimulate expression of ODF mRNA, OCIF genes, and Toll like receptors (TLR) present on the osteoblasts surface. After blocking the TLR, no ODF mRNA expression was observed. This observation supports the role that LPS and TLR play in the destruction of bone tissue [17]. Mogi *et al*. have assessed the RANKL/OPG level in gingival crevicular fluid in patients with periodontitis. They have stated the negative correlation between RANKL and OPG concentration [18]. The RANKL/OPG proportion was significantly higher in the periodontitis group as compared to the healthy control. This study has supported the hypothesis, that exogenic osteoprotegerin stimulates trabecular bone growth, inhibits osteoclastogenesis, diminishes the activity of mature osteoclasts and induces their apoptosis. Moreover in mice lacking osteoprotegerin expression an increased osteoclasts formation and bone resorption can be observed [19]. The immunomodulatory processes also involve dendritic cells and defensines. The group of dendritic cells can be further divided into two distinct populations. The first group is of medullary origin. These cells are present in the skin (also known as Langerhans cells) and first of all in the T zones of lymph nodes and spleen. They belong to the same line as the macrophages and are distinctively active as the antigen presenting cells. The other type is also referred to as follicular dendritic cells. Their origin is not known. They can be found in the germinal centers of lymphoid tissue. These cells can present on their surface the antigen-antibody complexes for prolonged time. It was stated *in situ*, *in vitro*, and *in vivo* that the dendritic cells subpopulations exhibit different localization pattern in patients suffering from chronic periodontitis. The CD1a+ cells (immature Langerhans cells) were observed in the epithelium, while the mature dendritic cells occupied the lamina propria of oral mucous membrane. Moreover high number of Langerhans cells is also present in the healthy gingival epithelium [20]. According to the studies, these cells further differentiate upon stimulation by the cytokines and pathogens of the mucosal membrane and their number gradually increases in the course of periodontal tissue inflammation. Based on these observations, Jotvani *et al*. have developed a new pathophysiological model of chronic periodontitis natural history [21]. According to this hypothesis, the immature CD1a+ cells, subjected to antigens and cytokines, migrate to the lamina propria, where they differentiate into the CD83+ cells. These mature cells migrate to the lymph nodes, where they present antigens to the lymphocytes. The mature dendritic cells comprise oral lymphoid follicles (OLF) system. Defensines α and β (HNP and HBD) play a linking role between the innate and adaptive immune reaction in the etiopathogenesis of periodontitis. They comprise heterogeneic group of peptides of antimicrobial activity. They are mainly released by the neutrophils upon activation of the TLRs present in the neutrophil cell membrane by bacterial antigens. They can be found in body fluids and secretions, such as saliva, but also intracellulary, i.e. within the epithelium of gingival crevice. They play a protective role before the specific antibodies appear in place and also support the antibodies at the later stages of defense. The TLRs induce chemotaxis of CD4+ CD45RA+ cells (naive T cells), CD45RO+ cells (T helper cells), CD8+ cells, and immature dendritic cells. Moreover they stimulate TNF-α and IL-1 secretion by monocytes [22]. The *in vitro* studies have also confirmed antimicrobial activity of defensins against *P. gingivalis*, *A. actinomycetemcomitans*, *S. gordonii*, *S. mutans*, and *C. albicans*. Synthetic analogues of defensins, administered intranasally, have been shown to induce immune reaction and thus can be used in the prophylaxis and treatment of periodontal disease [23, 24]. One of the factors that modify the inflammatory response in periodontitis is acute phase proteins, such as fibrinogen. Fibrinogen is not only a coagulant, but also a multidirectional immunomodulator. It is a ligand for the surface neutrophils receptors CD11b/CD18 and CD11c/CD18, thus influencing the chemotaxis, adhesion, and fagocytosis; it can also regulate the expression of IL-1β gene. According to a study performed by Sahingur *et al*. there is a correlation between the polymorphism of β-fibrinogen encoding gene at base 455 G/A and the chronic periodontitis [25]. By means of the Restriction Fragments Length Polymorphism (RFLP) the authors have proven a significantly higher fibrinogen concentration in the sera of patients homozygotic for A455 allele (H2H2 genotype) as compared to the H1H1 and H1H2 genotypes. Moreover the fibrinogen level in periodontitis patients was significantly higher than in the

healthy control. Another factor modulating the immune response is fractalkine (CX3CL1) – a chemokine belonging to the complement system. Hosokawa *et al*. have used polymeraze chain reaction (PCR) to prove the expression of CX3CR1 (fractalkine receptor) mRNA in the leukocytes infiltrating periodontal tissue [26]. Fractalkine binds to the receptor, stimulating migration of the leukocytes to the inflammatory focus. The lipopolysaccharide (LPS) of P. gingivalis has been proven to regulate fractalkine synthesis in epithelial cells. In the recent papers much attention has been paid to the reactive oxygen species (ROS) and their role in the inflammatory processes within periodontal tissue. The inflammatory cells, mainly polymorphonuclear leucocytes (neutrophils, monocytes, macrophages) exhibit increased metabolic rate, respiratory burst, as well as free oxygen radicals and reactive oxygen specias formation. These partially reduced oxygen products take part in microbial destruction, yet when present in high concentration they lead to toxic periodontal tissue damage by degradation of proteins. The mechanism of protection against these processes depends of antioxidants suppressing the free radicals formation, such as dismutase, peroxidase, myeloperoxidase, catalase, lactoferrin, S-transferase, transferrin, ceruloplasmin, and glutathione, as well as antioxidants eliminating the free radicals, such as albumin, bilirubin, uric acid, and ubichinone [27, 28]. Wei *et al*. have observed increased concentration of peroxidase, lactoferrin, and myeloperoxidase in the gingival crevicular fluid in chronic periodontitis as compared to the control group [29]. Moreover the concentration of these three antioxidants correlated significantly with clinical periodontal parameters: plaque index, pocket depth, and clinical attachment loss. Also the concentration of proteolytic enzymes increases in the gingival crevicular fluid and in saliva in the course of periodontitis. Periopathogenic bacteria, polymorphonuclear cells, as well as other immune system cells secrete matrix metalloproteinases (MMPs) – enzymes playing crucial role in degradation of extracellular matrix. Their activity is diminished by their natural, endogenic inhibitors: TIMPs (tissue inhibitors of metalloproteinases). Another protease that digests collagene and other gingival structures is human leukocyte elastase (HLE). The endogenic protection against the uncontrolled HLE functioning is the leukocyte protease inhibitor. The protease/inhibitor imbalance is one of the mechanisms of destructive processes in periodontal tissue [30].

The research directions in the field of immunologic aspects of periodontitis, pointed out in this paper, clearly indicate the complexity of the pathological processes, leading to destruction of periodontal tissue. The results published to date suggest that not all the pathogenic factors disturbing the homeostasis of periodontal complex have already been fully elucidated. There are still many problems that require further extensive research.

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