Review paper

Cytokines and periodontitis. Part I: interleukin-1 and interleukin-1 receptor antagonist

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Abstract

Studies on periodontal disease pathogenesis are for years concentrating on elucidating the mechanism of immune reaction to endotoxins, exotoxins and other products of bacterial cell metabolism. The course and severity of periodontitis can be significantly affected by bacterial virulence as well as host immunity dysfunction. Periodontal tissue destruction has been proved to result from cascade of cytokines synthesized by reactive cells upon stimulation by pathogenic bacteria and lipopolysaccharides within their cell membranes. Trials are undertaken to inactivate interleukin-1 (IL-1) – the most active cytokine in the pathogenesis of periodontal disease. Much hope is placed in gene therapy, utilizing modified techniques of gene transfer into the target cells. The clinical use of genetically programmed cells, producing substances blocking IL-1, based on recombinant IL-1 antagonist, as well as cytokines activating fibroblasts and osteoblasts to regenerate the destroyed periodontal tissue could prove alternative to the conventional treatment.

Key words: *periodontitis, host response, interleukin-1, interleukin-1 receptor antagonist.*

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Maturation, activation and migration of immune cells in the inflammatory environment is only possible thanks to the signaling glycoproteins – cytokines. Cytokines are released both by the local cells and by the migrating leukocytes. To transmit a signal to the target cell, the cytokine needs to be recognized by this cell. This process involves binding to the specific receptor. The extra-membranous fragment of this receptor is located on the target cell surface. The specificity of receptor means it can only bind to one type of cytokine. Some cells express receptors of low specificity, leading to competitive cytokines binding. The cytokine receptors can be divided into four main classes: type-1 – receptors containing own tyrosine kinase, type-2 – receptors acting by activation of intracellular tyrosine kinase, belonging to other structures, type-3 – serpentine receptors and type-4 – receptors containing serine/threonine kinase. This classification is based on receptor structure as well as the pathway of transmitting signal to the cell nucleus. Regardless of the receptor structure, its proper function requires three elements: extracellular domain, capable of cytokine binding; transmembrane domain, transmitting signal induced by cytokine binding into the cell, and intracellular system, transmitting this signal to the cell nucleus. Incapacity of either of these elements leads to lack of cellular reaction to the signaling cytokine. By participating in the communication between immune system and inflammatory process, the cytokines act mainly locally and exhibit high focal concentrations, simultaneously presenting low level or being undetectable in blood [1]. The cytokine concentration in biological material has been proven to depend on individual factors, such as localization and diagnosis. Some of the glycoproteins, such as interleukin-6 (IL-6), exhibit in case of inflammatory state, injury or infection increase of concentration, detected not only locally, but also in the peripheral blood and in the cerebrospinal fluid. Cytokines can also act like hormones. The expression of proteins building cytokine receptors usually antecedes specific effector mechanisms, initiated within target cells to combat pathogens. Interleukin-1, IL-4, IL-6, IL-8 and tumor necrosis factor α (TNF-α) have been shown to play crucial role in the induction and modulation of effector mechanisms in case of periodontal disease [2].

The quantitative and qualitative studies of IL-1, IL-1 receptors and inhibitors seem to have significant diagnos-

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tic and prognostic value for the initiation and progression of periodontitis. Interleukin-1 is a glycoprotein of molecular weight of 17 kDa. It is expressed by monocytes, macrophages, endothelial cells, lymphocytes, fibroblasts, keratinocytes, Langerhans cells and glial cells. It is also one of the main regulators of inflammatory and immunologic processes. Interleukin-1 release in periodontal tissue is mainly induced by lipopolysaccharides of Gram-negative plaque bacteria, as well as viruses, yeasts, exotoxins, peptidoglycans, complement component C5a and also IL-2, IL-3, IL-12 and IL-1 itself. There are two distinct IL-1 forms identified: IL-1α and IL-1β. They are produced in the effect of two different genes expression and they bind to different receptors on different target cells. The homology between these two forms equals 25%. They are synthesized in the cell in form of precursors, which are then submitted to the action of specific proteases. Yet IL-1α is already active in the precursor form. In case of IL-1β the inactive precursor is the substrate for cysteine protease (ICE). Active form of IL-1α, as opposed to IL-1β, stays connected in a respective ratio to the cell that it was synthesized by. It is present both inside and on the cell surface. This cytokine is characterized by broad spectrum of biological activities. It simplifies the inflammatory process development, influencing the neutrophiles and monocytes, increases prostaglandins and leukotriens synthesis and secretion, increases leukocytes adhesion to the endothelial cells, stimulates fibroblasts and keratinocytes proliferation, promotes platelet-derived growth factor (PDGF) and plaque activating factor (PAF) secretion as well as activates macrophages and osteoclasts, thus being involved in the periodontal tissue destruction. Moreover IL-1 induces gene expression of the major histocompatibility complex on T- and B-cells, leading to their activation and immunoglobulin synthesis. It exerts biological effect on the other cytokines. In cooperation with antigens, it induces IL-2 synthesis as well as the expression of IL-2 receptor. Moreover it promotes IFN-γ and IL-6 synthesis. In course of periodontal disease a synergism between IL-1 and other pro-inflammatory cytokines can be observed [3]. There are two IL-1 receptor forms identified: IL-1 RI (80 kDa) and IL-1 RII (68 kDa), both belonging to the cytokine receptors type 4. They are characterized by extracellular immunoglobulin-like domains, connected to the intracellular fragment, similar to fibronectin III. The first type of receptor, present on most of the cells sensitive to IL-1, is responsible for induction of signal transmission into the cell. As opposed to type I, IL-1 RII is mainly expressed on neutrophils, monocytes and B-cells. It cannot transduce signals and most probably acts as a safety guard in case of excessive IL-1 amounts in the inflammatory site. Binding of IL-1 by the IL-1 RI leads to phosphorylation of I κB α and activation of NF-κB protein. NF-κB belongs to the family of cytoplasmic transcription factors, that bind directly to the specific promoter site of variety of genes. Alternative way of transmitting the signal by IL-1/IL-1 RI complex is induction of cascade of subsequent cytoplasmic transcription factors activation. These factors are Ras, Raf, Mek and nuclear factor MAPK [4]. By stimulating the immune and inflammatory reaction, IL-1 activates natural immunosuppresive mechanisms, protecting host from excessive, uncontrolled progression of this reaction. It affects hypothalamus, increasing release of corticoliberin and thus stimulating synthesis of corticotropin and, as a result, glucocorticosteroids.

A specific natural protein of 22-25 kDa molecular weight has been described, acting as an IL-1 receptor antagonist. Explaining of the precise function of IL-1 receptor antagonist (IL-1Ra) as well as other endogenous and genetically modified IL-1 inhibitors could contribute to the clinical use of this knowledge. The first trials have been undertaken to administer IL-1Ra to the patients suffering from rheumatoid arthritis, ulcerative colitis, as well as septic shock. A gene therapy has been also introduced, basing on introducing IL-1 receptor antagonist gene (IL1RN) to achieve its local synthesis. Human IL1RN gene maps to the long arm of chromosome 2. It is also a region for the IL-1β and IL-1 receptors I and II loci. Yet the meaning of sharing the same chromosomal localization by three distinct IL-1 forms as well as two receptor types in humans is not clear. It has been hypothesized, that the difference between IL-1 and IL-1Ra results from partial duplication of inherited IL-1 gene [5].

Some early studies on IL-1Ra dealt with biological activity of the filtrate of human monocytes, cultured in presence of adherent immunoglobulin G, as well as with urine of patients suffering from myelomonocytic leukemia. The subsequent studies revealed characteristics of a recombinant IL-1Ra [5]. The assessment of IL-1Ra concentration in blood and other body fluids, as well as relations between them, *in vitro* and animal surveys as well as studies on genetic modification on this cytokine inhibitor allowed for clinical trials on practical cytokine administration in blood vessels inflammatory disease, vein thrombosis, osteoporosis, glomerulonephritis, diabetes and autoimmune disorders [6]. Lipopolysaccharide (LPS), some cytokines [IL-4, IL-10, granulocyte-macrophage colony stimulating factor (GM-CSF) and transforming growth factor-β (TGF-β)], as well as various immune complexes can stimulate expression of IL-1 receptor antagonist, capable of binding to both IL-1 receptor types. Dinarello claims the most frequent stimulus promoting the secretion of this specific protein are the bacterial endotoxins [7]. Stimulated cells, such as polymorphonuclear leukocytes (PMN), can activate IL1RN gene expression and protein translation [8]. Interleukin-1 receptor antagonist blocks IL-1 activity, both *in vivo* and *in vitro*. Recombinant IL-1Ra inhibits IL-1-promoted thymocyte and chondrocyte proliferation, as well as collagenase, GM-CSF and IL-6 synthesis [9]. Rambaldi *et al.* have reported high IL-1Ra activity in the reduction of leukaemic cells proliferation [10]. Interleukin-1 receptor antagonist administration to the rabbits suffering from intestinal inflammation

reduces inflammatory infiltration and gut swelling [11]. Intravenous injections of *Escherichia coli* in rabbits trigger septic shock, characterized by hypotension, intravascular immunity deficiency, leukopenia and thrombocytopenia. Upon IL-1Ra administration only mild, transient hypotension was observed [12].

Studies on periodontal disease pathogenesis are for years concentrating on elucidating the mechanism of immune reaction to endotoxins, exotoxins and other products of bacterial cell metabolism [13, 14]. The course and severity of periodontitis can be significantly affected by bacterial virulence, as well as host immunity dysfunction: "overreaction" as well as diminution of repair functions [15-17]. Culture supernatant has been proven to hydrolyze IL-1β, IL-6 and IL-1Ra. The severity of periodontitis is commonly thought to depend on the quantity of *Porphyromonas gingivalis* colonies. The presence of this species significantly debilitates IL-1Ra anti-inflammatory action. As stated above, IL-1Ra can take part in biofeedback mechanism; thanks to the structural similarity to IL-1 it can bind to IL-1 RI and IL-1 RII receptors. According to one hypothesis, IL-1Ra production is a part o regulatory host reaction, aiming to diminishing of pro-inflammatory IL-1 activity [18]. Periodontal tissue destruction has been proved to result from cascade of cytokines synthesized by reactive cells upon stimulation by pathogenic bacteria and LPS within their cell membranes. The molecular studies did not by far explain, if different clinical forms of periodontal disease correlate with differentiated secretion of cytokines by the stimulated cells. The *in vitro* and *in vivo* studies suggest that the proportion of pro-inflammatory and anti-inflammatory cytokines could influence in a significant way the course of pathological processes within periodontal tissue. Interleukin-1 receptor antagonist present in concentration exceeding the IL-1β level by 100- or 1000-fold can inhibit the IL-1 activity [19]. The development and progression of periodontitis is not only influenced by IL-1 to IL-1Ra ratio, but also by other cytokines, pro-inflammatory prostaglandins and oxygen radicals [20, 21]. According to Kabashima *et al.*, the presence of IL-1Ra in gingival crevicular fluid is also a hallmark of inflammatory process: the authors have not identified IL-1Ra in patients free from periodontal disease [22]. Holmlund *et al.* assessed IL-1Ra concentration in gingival fluid collected from periodontal pockets before and after treatment. In material obtained from patients with chronic periodontitis the concentration of IL-1Ra was significantly higher, as compared to the control group; moreover a remarkable decrease of IL-1Ra level was observed after treatment [23]. Thus it could be deduced that the clinical improvement is accompanied by IL-1Ra concentration downturn. Nishihara *et al.* have proved in a study on mice that IL-1Ra released by cells upon stimulation by LPS of *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans* inhibits bone tissue resorption [24]. Similar results were also reported by Oates *et al.*, as well as Rasmussen *et al.* [25, 26]. Delima injected human recombinant cytokine IL-1Ra into interdental papillae in monkeys with experimentally induced periodontitis. Upon treatment the alveolar bone resorption has been proved to be decreased by 91%, while clinical attachment loss was diminished by 51% [27]. Yet Ishihara *et al.* and Rawlinson *et al.* did not support the results presented above [28, 29]. According to these authors the concentration of IL-1Ra in crevicular fluid of patients suffering from periodontal disease was decreased as compared to the control group exhibiting no clinical signs of periodontitis. It could suggest protective role of IL-1Ra, supporting the hypothesis, that in the periodontitis group the concentration of inhibitor was to low to constrain the inflammatory process. The studies performed by Rawlinson suggest a reverse correlation between IL-1α and IL-1Ra concentration in gingival fluid of patients suffering from periodontitis. It should be noted that the intracellular form of IL-1Ra can only act when released from dead or necrotizing tissues together with IL-1β (in case of macrophages) and IL-1α (in case of keratinocytes) [29]. The subsequent studies by Rawlinson *et al.* have supported the previous observation of significantly decreased IL-1Ra concentration in gingival crevicular fluid in patients suffering from periodontitis as compared to the control group, presenting no clinical signs of periodontal disease [30]. Waschul *et al.* measured the IL-1Ra and IL-1β concentration in gingival fluid in case of experimentally induced gingivitis. They did not observe a significant difference of these cytokines concentration, probably suggesting, that the increase of pro-inflammatory cytokine is not compensated by sufficient inhibitor secretion [31]. Own studies, carried out for many years, did not reveal any differences between IL-1Ra salivary concentration and periodontal pockets depth in patients with periodontitis [32]. Insufficient IL-1Ra release in response to pro-inflammatory factor leads to the development and exacerbation of inflammatory processes within periodontal tissue. Interestingly, high concentrations of IL-1Ra have been detected in amniotic fluid, suggesting that a healthy organism is capable of high local synthesis of this inhibitor [5]. Multiannual own studies have proved the presence of IL-1Ra in saliva in patients with clinically healthy periodontal tissue [32]. In some cases this glycoprotein may not act as a cytokine inhibitor in course of inflammatory process within periodontal tissue. The periodontal pathogens can influence the cytokine network within periodontal tissue and gingival crevicular fluid, blocking the activity of the inhibitors. Fletcher *et al.* have stated that the anti-inflammatory activity of IL-1Ra can be decreased by bacteria *Porphyromonas gingivalis*, that can hydrolyze described cytokine [21]. On the other hand, Schytte Blix *et al.* have proved, basing on *in vitro* and *in vivo* studies, that the course of pathogenic process in periodontium depends on the proportion of pro-inflammatory and anti-inflammatory cytokines [19]. Only extremely high IL-1Ra concentration, significantly exceeding the IL-1β concentration,

could inhibit the biological activity of this cytokine. The results published by Schytte Blix *et al.* have been supported by other authors. It was observed (in the *in vitro* studies) that to decrease by 50% prostaglandins and collagenase synthesis, which is induced both by IL-1α and IL-1β, the IL-1Ra concentration should overwhelm the pro-inflammatory cytokine by over 100 times. This observation has been explained by the "spatial receptor effect". Target cells can present a couple thousands of receptors, and yet even much smaller amount of the IL-1 receptors would be enough to induce regular biological response [5]. Yoshinari *et al.* were the first ones to mention the total interleukin-1 activity. It is calculated as a product of IL-1α, IL-1β and IL-1Ra concentration in gingival fluid. The highest values of this index were observed in patients with peak alveolar bone reduction. Moreover a positive correlation between this index and clinical parameters, as well as its decrease after treatment were observed [33]. Thus the *in vitro* and *in vivo* studies suggest that the proportion of pro-inflammatory and anti-inflammatory cytokines could influence in a significant way the course of pathological processes within periodontal tissue. Interleukin-1 receptor antagonist present in concentration significantly exceeding the IL-1 level can inhibit the cytokine biological activity. In conclusion, the pro-inflammatory or anti-inflammatory environment within the tissues affected by periodontitis depends on multiple factors. This fact influences the therapeutic process. Trials are undertaken to inactivate interleukin-1 – the most active cytokine in the pathogenesis of periodontal disease. Much hope is placed in gene therapy, utilizing modified techniques of gene transfer into the target cells [34]. The clinical use of genetically programmed cells, producing substances blocking IL-1, based on recombinant IL-1 antagonist, as well as cytokines activating fibroblasts and osteoblasts to regenerate the destroyed periodontal tissue could prove alternative to the conventional treatment. New potential possibilities of periodontal disease treatment have arosen in 2002, when Anakinra was registered. The active substance of Anakinra is IL-1Ra. It has been registered for rheumatoid arthritis treatment [35, 36].

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