Review paper

Cytokines and periodontitis. Part II: tumor necrosis factor and soluble tumor necrosis factor receptor I

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Abstract

Quantitative and qualitative studies concerning tumor necrosis factor α (TNF-α) and its receptors can prove essential for periodontal diseases diagnostics. Tumor necrosis factor is believed to be a very important mediator of inflammatory reactions and host immune response. Tumor necrosis factor α is the key cytokine of local inflammatory reaction within periodontal pocket, resulting from stimulation by bacterial factors. This cytokine initiates inflammatory process and activates mechanisms leading to periodontal tissue destruction. Another cytokine of interest in respect to periodontitis ethiopathogenesis is soluble TNF receptor I (sTNF RI). Observation of soluble TNF receptors as physiologic inhibitors of TNF led to its administration in therapeutic process as well as in therapy selected cases of aggressive periodontitis.

Key words: *periodontitis, host response, immune system, tumor necrosis factor (TNF), soluble tumor necrosis factor receptor I (sTNF RI).*

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According to the commonly accepted, basic model of cell interactions, the regulatory cells secret to the environment cytokines, that migrate freely through the extracellular fluid until they reach the target cells, where they bind to the surface receptors, transmitting signal to the cell nucleus. Such an interaction between cells doesn't require physical contact between them. At the same time cytokine synthesis by the regulatory cells changes the activity of gene(s) within the regulated cells, influencing their functional state. Cytokines can also be membrane bound, with no possibility to be released to the environment. Such a situation also allows for transmitting signal by the cytokine and receptor into the cell nucleus, but it requires physical contact between regulating and regulated cells. In normal situation such a contact takes place for example within bone marrow, where the cells of haematopoietic microenvironment adhere to the young cells, that will develop to the mature blood cells. One can also distinguish free receptors, unbound to the cells producing them (lacking transmembrane and intracellular domain), so called "soluble" receptors. They are formed by proteolytic degradation of membrane receptors or by translation of pre-mRNA lacking transmembrane domain. Such the "crippled" receptors are produced deliberately and take part in regulating cell functions. One of the activities they can play is competition with the membrane receptors, i.e. binding and blocking the cytokines. It could be called antagonistic action. Another possibility is signal reinforcement by the cooperation with membrane receptor, when the complex of soluble receptor and cytokine activates the membrane receptor. The basic difference in comparison to the other models is lack of possibility to transmit signal into the cell nucleus if a cell only produces soluble receptors [1]. Quantitative and qualitative studies concerning tumor necrosis factor α (TNF-α) and its receptors can prove essential for periodontal diseases diagnostics [2, 3].

Tumor necrosis factor is believed to be a very important mediator of inflammatory reactions and host immune response. Tumor necrosis factor belongs to the protein superfamily, comprising: TNF, lymphotoxin α (LT-α), LT-β, TRAIL (APO-2L) and ligands for CD27, CD30, CD40, CD95 (APO-1/Fas), 4-1BB and OX40. Tumor necrosis factor together with other cytokines (some of them TNF-induced) affects monocytes, macrophages, fibroblasts, neutrophiles, keratinocytes and mastocytes in an endocrine, paracrine and autocrine way. First of all TNF leads to the haemorrhagic necrosis of solid tumors. Not only it dam-

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ages endothelium of tumor blood vessels, but also influences development, differentiation and function of many cells, including tumor cells and normal cells. Tumor necrosis factor activates natural killer cells (NK-cells), promotes phagocytic activity of neutrophiles, increases proliferation and differentiation of T- and B-cells, induces interleukin 1 (IL-1) synthesis and increases its expression on the surface of epithelial cells. It increases the capacity of endothelial cells to accept lymphocyte adhesion, stimulates fibroblasts growth, as well as regulates collagen, fibronectin and hyaluronic acid synthesis within the fibroblasts. Moreover TNF induces collagenases and other proteases synthesis, as well as activates osteoclastogenesis, osteoclasts maturation and bone resorption. It plays a role of local moderator of bone remodelling in case of inflammation [4-6]. There are two distinct forms of TNF: TNF-α (or cachectin) and TNF-β, called lymphotoxin (also present in two forms, α and β). Recently this nomenclature, distinguishing TNF-α and TNF-β, is getting out of use. Cachectin is simply called TNF, while for TNF-β the original term LT is used. Genes encoding TNF are the only cytokine genes mapping together with the major histocompatibility complex. They are localized to the short arm of chromosome 6, between p23 and q12. Each of the two genes comprises four exons and three introns. Messenger RNA for TNF includes repeated octamer sequence (UUAUUUAU)n, localized in the 3' untranslated region. This sequence is also present in mRNA for other cytokines, such as IL-1, interferons (IFN), as well as some oncogenes. Promoter region for TNF exhibits polymorphism. Tumor necrosis factor precursor is a cell membrane protein, released to the environment upon digestion by protease. Biologically active TNF presents in form of trimers stabilized by non-covalent interactions. Tumor necrosis factor trimers consist of identical chains (homotrimers). Transmembrane TNF, a precursor for soluble TNF, is also biologically active. It plays its role via TNF receptors. While the membrane form of cachectin is a precursor for soluble one, the membrane bound and soluble forms of lymphotoxin differ. Both membrane and soluble one present in form of trimers, similarly to cachectin. Yet only the soluble form (LT-α) is a homotrimer like cachectin (consists of three identical α chains). The membrane bound form, known as LT-β, comprises one α chain and two β chains or two α chains and one β chain. Tumor necrosis factor is secreted by immune system cells upon stimulation by variety of factors, such as lipopolysaccharide (LPS) of bacterial cell wall (endotoxins), lymphokines, viruses, parasites, other cytokines (IFN-γ, IL-1, TNF alone in an autocrine way) and tumor cells, as well as some pharmaceuticals, such as phosphatase inhibitors, cyclooxigenases or benzodiazepins. Lipopolysaccharides of bacterial cell walls are responsible for increase of TNF production in bacterial infection. In acute state it can lead to the septic shock, multiple organ dysfunction syndrome and death, while in chronic infections it could eventually lead to cachexy. T-cells (both CD4+ and CD8+) and B-cells produce lymphotoxins, but they can also secrete small amounts of TNF. Tumor necrosis factor secretion is inhibited by other cytokines: transforming growth factor β (TGF-β), IFN-α, IFN-β, IL-4, IL-6, IL-10, IL-11 and IL-13, Epstein-Barr virus, as well as some pharmaceuticals (e.g. phosphodiesterase inhibitors), metaloproteinases, lipooxygenases, prostaglandin E2, estradiol, progesterone [7, 8]. Tumor necrosis factor activation depends on specific cell receptors. There are two types of TNF receptors present on cells: TNF RI (CD120a, 55 kDa) and TNF RII (CD120b, 75 kDa). Both receptor types are glycoproteins. Their extracellular domain is similar to the corresponding part of nerve growth factor (NGF) receptor. Tumor necrosis factor RI and TNF RII belong to the protein superfamily together with CD27, CD30, CD40, CD95, 4-1BB, OX40, DR-3, DR-4 and DR-5. Characteristic feature of these proteins is presence of various amounts of cysteine-rich domains of about 40 aminoacids. Their activity and signal transmitting potential differs. Tumor necrosis factor RI presents higher activity. Tumor necrosis factor receptor p55 has been observed to play main role in promoted by TNF-α up-regulation of integrine receptors on neutrophils. Each of the receptors binds both TNF-α and TNF-β (LT-α and LT-β), while both lymphotoxin-β forms bind also to additional cell receptor TNF RIII. Tumor necrosis factor receptors are present on nearly all the nucleated cells in mammals. Some cells, such as fibroblasts, lymphocytes or endothelial cells, present both receptors for cachectin and for lymphotoxin. No correlation has been observed between number of receptors on cell surface and its reaction to TNF. Tumor necrosis factor receptor expression on cell surface is up-regulated by IL-1, IFN-γ, IL-2, as well as TNF itself. Paśnik *et al.* cite studies by Della Bianca *et al.*, according to which both p55 and p75 take part in phagocytosis. Signal transmitting requires p55 receptor. Specific binding between ligand and this receptor depends, by contrast, on p75 receptor [9, 10]. In human blood soluble TNF receptors, so called TNF-binding protein, can be periodically detected. They are extracellular fragments of membrane receptors, produced by specific enzyme action. In human serum and urine two receptors were identified: TNF-R55-BP and TNF-R75-BP. Soluble TNF receptors are still capable of cytokine binding, acting as competitive inhibitors of membrane receptors. Upon receptor separation the cell loses possibility of signal receiving. Soluble TNF receptor in low concentration can can exhibit TNF-agonistic activity by active trimer stabilization and preventing its dissociation to inactive monomers. In high concentration, on the contrary, it acts in an antagonistic way, binding excessive amounts of cytokine at the site of inflammation. I can be perceived as a kind of biofeedback [11-13]. Increased soluble receptor concentration is observed in patients suffering from neoplasms, acquired immunodeficiency syndrome (AIDS), autoimmune disorders, as well as transplant recipients exhibiting rejection syndrome.

Activated cell, e.g. macrophage, produces broad spectrum of cytokines. The cell receptors on the other hand show affinity for various cytokines. In the cytokine complex within pathological spot each cytokine can both induce and inhibit other cytokines. Soon after pro-inflammatory cytokines release, also anti-inflammatory cytokines are secreted, leading to the limitation of disease spreading, both in time and space. Tumor necrosis factor influences various target cells, thus presenting a strong mediator of inflammatory and immune processes. Many studies were performed, aiming at monitoring of TNF concentration in periodontal tissue, gingival crevicular fluid and blood. According to Matsuki *et al.*, TNF-α is the key cytokine of local inflammatory reaction within periodontal pocket, resulting from stimulation by bacterial factor [14]. This cytokine initiates inflammatory process and activates mechanisms leading to periodontal tissue destruction. According to these studies, TNF-α plays a role of fire alarm and coordinator of cytokine response. Tumor necrosis factor α induces chemokines and adhesive molecules, thus leading to the fast immune response and leukocyte migration to the gingival sulcus. Fibroblasts and osteoclasts stimulation and expression lead to periodontal membrane and bone tissue degradation [14]. Stashenko *et al.* have demonstrated significant decrease in pro-inflammatory cytokine concentrations after periodontal treatment, thus suggesting the correlation with severity of pathological process [15]. Bacteria present in periodontitis can lead to so called focal infection, presenting for example as endocarditis, arthritis or rheumatic disease. According to Beck *et al.* a common pathomechanism could be activated in this situation, including genetic monocyte hyperactivity. Upon endotoxin stimulation, these monocytes would secrete pro-inflammatory cytokines. That would lead to overexpression of adhesive molecules and immune reaction dysregulation [16].

Another cytokine of interest in respect to periodontitis ethiopathogenesis is soluble TNF receptor (sTNF RI). According to the own studies there was no significant difference between sTNF RI salivary concentration in chronic periodontitis and healthy control [17]. Probably the initial upregulation of receptor secretion, stimulated by growing TNF concentration, is short-lasting and rapidly returns to baseline. Statistical analysis has not revealed any correlation between sTNF RI concentration and clinical parameters of periodontal disease. It could be hypothesized, that the soluble form of receptors play a role of physiologic cytokine inhibitors. Lack of significant difference in sTNF RI concentration between periodontitis and health control could result from decreasing role of physiologic inhibitors and dysregulation of cytokine production in chronic phase of periodontal disease. Lilic *et al.* examined the role of cytokines in oral candidiasis in an *in vitro* study. They did not observe increased secretion of sIL-6R (soluble IL-6 receptor), even though IL-6 plays an important role in *Candida albicans* infection. Their results suggest that low concentration of receptor could prevent excessive IL-6 activity [18]. The literature on cytokine penetration from gingival crevicular fluid to saliva is scarce. Soluble tumor necrosis factor receptors are observed in serum and urine of healthy people. Their concentration increases in various bacterial and viral infections, such as acquired immunodeficiency syndrome (AIDS), as well as in neoplasms [19]. McFarlane *et al.* reported positive correlation between TNF and soluble TNF receptor concentration in the peripheral blood of periodontitis patients [20]. Tervahartiala *et al.* examined TNF-α and TNF receptors p55 and p75 in periodontal tissue inflammation. They observed positive correlation between the expression of TNF-α p55 receptor and the severity of periodontal pocket infiltration by macrocytes, macrophages, fibroblasts and endothelial cells. Conversely, p75 receptor is only occasionally detected. In this situation, characterized by increased TNF-α+ epithelial cells number and p55 level, the collagenolytic activity of metaloproteinases also increases. Presumably this receptor plays important role in osteolysis induction, while p75 receptor takes part in cell differentiation at the early stages of haematopoiesis. Perhaps the dysfunction of tissue enzymes, activating membrane receptors, is one of the reasons explaining lack of significant difference between sTNF RI in chronic periodontitis and in control group [21]. Graves *et al.* have proven significant mitigation of inflammatory cells infiltration when a human monoclonal anti-TNF antibody was administered to the animals with experimentally induced periodontitis. Moreover the distance between "inflammatory front" and alveolar bone surface increased. Osteoclasts were scarcely present in analyzed specimens, in contrary to the control group that received no antibodies. Studies on polymorphonuclear cells (PMN) revealed impaired chemotaxy, migration and phagocytic activity as well as a significant increase in surface receptor expression [22]. In case of periodontal disease, dysfunction of PMN cells leads to rapid progression of disease as a result of proteolytic enzyme activity. One of these enzymes, elastase, is intimately destructive for periodontal connective tissue. It activates pro-inflammatory cytokines as well. Cope *et al.* stated that the concentration of soluble TNF receptor in blood of patients suffering from arthritis was growing parallel to the disease severity. This observation led to utilization of above-mentioned receptor as a marker in inflammatory process monitoring [23]. Observation of soluble TNF receptors as physiologic inhibitors of this pro-inflammatory cytokine led to its administration in therapeutic process [24-27]. Etanercept is a commercially available drug, synthesized by p75 receptor and Fc component of immunoglobulin G fusion. It binds to TNF-α, thus exerting competitive activity against this cytokine. Similarly, infliximab is an IgG1 antibody against TNF-α. Its activity results from blocking the binding sites of TNF-α receptor. Adalimumab is monoclonal IgG1 antibody, blocking in the inflammatory environment the TNF receptors type I (p55)

and type II (p75) on inflammatory cells. The specific recombination of p55 receptor led to synthesis of pharmaceutical called lenercept. Other drugs used in rheumatoid arthritis treatment are monoclonal anti-TNF antibodies: human D2E7 antibody and chimeric Remicade, comprising mouse Fv and IgG1. Remicade has been originally synthesized for rheumatoid arthritis treatment, but it could probably prove beneficial in Crohn disease as well [28-30]. Another drug, called golimumab (human anti-TNF-α monoclonal antibody), is the first one registered in North America to be used in three indications: rheumatoid arthritis, psoriatic arthritis and ankylosing arthritis [31]. Mentioned above examples of TNF inhibitors utilization in treatment and monitoring of many chronic diseases could also lead to modern therapy development to be used in selected cases of aggressive periodontitis.

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