Evaluation of mCD14 expression on monocytes and the blood level of sCD14 in patients with generalized aggressive periodontitis

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Abstract

Purpose: **Lipopolysaccharides (LPS), a major component of the cell membrane of gram-negative bacteria, are the main stimulants of the host immune response, initiating inflammatory changes and responsible for periodontal tissue destruction. The mCD14, which is found primarily on monocytes and macrophages, is the key membranous receptor involved in LPS binding. CD14 is also present in the serum as a soluble form (sCD14) released due to shedding from monocytes.**

The aim of the study was to assess CD14 expression on peripheral blood monocytes in patients with generalized aggressive periodontitis (GAP). The level of sCD14 was also determined in the serum of GAP patients.

Material and methods: **The study group consisted of 16 patients with generalized aggressive periodontitis, the control group had 13 systemically and periodontally healthy subjects. The expression of mCD14 was determined by flow cytometry and expressed as mean intensity of fluorescence (MIF). Serum sCD14 level was examined with ELISA method.**

Results: **The expressions of mCD14 on monocytes in GAP patients and control subjects were comparable. No statistically significant differences were noted in the mean serum sCD14 level between GAP and control subjects.**

Conclusions: **As periodontitis is a local disorder affecting a small fragment of the oral cavity it seems likely that chronic bacterial infection existing there is not reflected in the peripheral parameters.**

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Introduction

Aggressive periodontitis (AP) is a particular type of periodontal tissue inflammation, characterized by rapid destruction of tooth-supporting tissues and leading to preterm dentition loss in young people. It is believed that this course of the disease can be caused by certain individual susceptibility and genetic phenotype predisposing to severe periodontal tissue destruction [1,2]. Periodontal destruction occurs due to spread of inflammation which originally acted as host defensive mechanism and was not suppressed at an appropriate moment [3].

Anaerobic gram-negative bacteria are the major etiological factor leading to periodontitis. Lipopolysaccharides (LPS), which are present in the cellular walls of the bacteria, act as the most important stimulants of the host immune response which initiates inflammatory changes and periodontal tissue destruction [4]. The mCD14 found primarily on monocytes and macrophages and more seldom on activated neutrophilic granulocytes (PMN) is the key membranous receptor responsible for LPS binding [5]. 90% of monocytes axhibit strong CD14 staining. These $CD14^{++}$ cells are CD16-negative simultanously. Another population of monocytes are double-positive $CD14^{+}CD16^{+}$ cells which show a low level of CD14 expression and strong CD16 expression [6]. CD14 is a 55 kDa glycoprotein (glycosol-phosphatidyl-inositol). LPS is bound to CD14 by LPS-binding protein (LBP) present in the blood serum [7]. LPS, TNFα and IFNγ cause an increase in CD14 expression on human myeloid cells in vitro, while IL-4 decreases it [8]. CD14 is also found in the serum as a soluble form (sCD14) that appears due to proteolytic cleavage and phospholipase D induced shedding from monocytes [8]. Binding of mCD14 to LPS induces a number of cell activities, including production of proinflammatory cytokines (IL-1, IL-6, IL-8, IL-12, TNFα) and anti-inflammatory cytokines (IL-10, TGFβ), as well as NO or oxygen radicals [9]. Most of these compounds affect alveolar bone

Table 1. **Morphological parameters of blood in AP and C subjects (mean ± standard deviation)**

	AP	C	p
WBC	6.1±1.3	5.35±1.2	p=0.74
MO%	8.3±2.9	6.3±2.9	p=0.12
MOan	0.5±0.2	0.5±0.3	p=0.54

WBC – white blood cells, MO% – % of monocytes; MOan – absolute number of monocytes

Table 2. **mCD14 on peripheral blood monocytes and sCD14 level in sera of GAP and C groups (mean ± standard deviation, minimum and maximum values)**

Parameter	GAP			C		
	mean	minimum	maximum	mean	minimum	maximum
mCD14	25.08±5.26	14.3	31.8	27.02±3.03	22.5	32.9
sCD14	1759±277	1294	2328	1796±165	1540	2006

resorption [4]. The role of sCD14 remains not fully elucidated. This molecule blocks binding of LPS by mCD14 and thus inhibits cell activation [8].

Immune system deficiencies towards the periodontopathogens have been reported in AP patients [1,3,10]. Therefore the question arises wheter abnormalities of mCD14 expressoin on blood monocytes and sCD14 serum concentration exists in AP patients and if these abnormalities can be involved in the etiology of aggressive periodontitis.

That's why the aim of the present study was to assess the mCD14 expression on peripheral blood monocytes and serum sCD14 level in patients with aggressive periodontitis.

Material and methods

16 generally healthy, no smoking GAP patients, aged 23--45 years (mean 37.5), 10 women and 6 men, were involved in the study. The control group (C) consisted of 13 periodontally healthy subjects, aged 25-47 years (mean 38.5), 8 women and 5 men. The subjects of control group were non-smokers who had not taken any drugs (including antibiotics) for the previous 6 months. The diagnosis was based on clinical and radiographic examination according to criteria of classification recomended by American Academy of Periodontology [11]. The control subjects had no history of periodontal disease (no bleeding on probing, probing pocket depth <3 mm, no attachment loss, lack of bone loss on radiographs). In GAP patients moderate or severe destruction of periodontal tissue were assesed. These patients had undergone rutine periodontal therapy (scaling and root planing, farmacological and surgical therapy) at least two years ago. All the study subjects gave their written consent for the collection of the research material. The study was approved by the Bioethics Committee of the Medical University of Białystok.

Blood for analysis was collected in the morning hours from the ulnar vein to a test-tube containing EDTA-K_3 as anticoagulant. Morphological parameters with full differentiation of peripheral blood leukocytes were assessed using a hematological analyzer Coulter MAXM (Coulter, USA). Results were expressed as absolute values (G/l) and percentage values (%). Monoclonal anti-CD14 antibodies marked with fluorescein isotiocianine (FITC) (DakoCytomation, Denmark) were used for study. Standard techniques of direct fluorescein labeling of whole blood leukocytes were used to assess antigen expression. 10μl of the monoclonal antibody was added to 100 μl blood and after 15-min. incubation at room temperature, with no access to light, the sample was processed by means of rapid lysis using EPICS IMMUNOLOGY WORK STATION with ImmunoPrep Reagent System. Following careful mixing, the sample was examined in a flow cytometer EPICS XL (Coulter) equipped with argon laser 488 nm. Each time, the apparatus was calibrated with DNA Check. Results were expressed as MIF – mean intensity of fluorescence, indirectly reflecting density of the molecule examined on cells. All the above tests were made from fresh samples of blood.

The ELISA method was applied to assess serum concentrations of sCD14, using a Quatikine Human sCD14 Immunoassay Kit (R&D, USA). Serum samples for ELISA analysis were stored at -80°C. Results were expressed in ng/ml.

Data were subjected to statistical analysis using Mann-Whitney test with SPSS 8.0 PL program. Statistically significant differences were considered at $p<0.05$. The Pearson test was applied to estimate the correlation.

Results

Mean morphological parameters (leukocytosis, % and absolute monocyte count) were similar in both groups (*Tab.* 1). Mean fluorescence value of mCD14 on monocytes in AP patients was comparable to control (p=0.28). No statistically significant differences were found in the mean serum sCD14 levels between GAP patients and control subjects (p=0.63). In both groups considerable individual differences in the parameters examined were observed. Numerical data referring to GAP and control groups have been presented in *Tab. 2*. There were no correlation found between expression of mCD14 and serum levels of sCD14 in GAP and control groups.

Discussion

The expression of mCD14 on monocytes and serum sCD14 level have been examined by many authors in various generalized inflammatory diseases. A significant increase has been found in these parameters in e.g. HIV-positive subjects, patients with acute vasculitis, systemic lupus erythematosus (SLE), sepsis, malaria and patients after hemodialysis [8,12-15].

In our study on the expression of mCD14 on monocytes no evident differences were noted between GAP and control subjects. At the same time, in both groups we found quite substantial differences in the parameters examined between the respective individuals. Our results seem to correspond with those of Buduneli et al. [16], who examined patients with various forms of periodontitis (P). These authors revealed no differences in the expression of mCD14 on monocytes between AP and control subjects. However, Shapira et al. [17] found a decrease in mCD14 expression on monocytes in AP patients.

In our study, also serum sCD14 level in GAP patients was similar to its level in healthy subjects. Hayashi et al. [18], however, found an increase in serum sCD14 level in patients with various forms of periodontitis. They noted that dental plaque LPS penetrating the gingiva had the most significant effect on sCD14 release. An increase in the plaque mass enhances the inflammatory reaction in periodontal tissue. With the progression of the inflammatory symptoms, bacterial products may get to the blood, thus stimulating sCD14 secretion by monocytes in P patients [18,19].

Also expression of this molecule after periodontal treatment was evaluated. The sCD14 concentration in patients after treatment was significantly decreased, reaching the level of healthy subjects [18]. This can partly justify our study outcome. Our patients had been previously treated (at least two years before the study) according to standard periodontal hygienic and surgical procedures, which brought considerable clinical improvement. In the study period, the patients had only slightly intensified symptoms of inflammation: gum swelling and bleeding from periodontal pockets during probing. It can be thus assumed that in AP patients serum sCD14 level can be close to the norm in the chronic phase of inflammation, though altered in the acute phase.

Other studies point at the likely relationship of CD14 with the pathomechanism of P. It has been found that the sCD14-LPS complex affects the activation of cells which originally showed no mCD14 expression, namely endothelial cells and fibroblasts of gingival tissue. Endothelial cells affected by this complex release inflammation mediators, whereas fibroblasts exhibit higher expression of intercellular adhesion molecule 1. On the other hand, sCD14 can play the role of carrier protein and transport LPS to high density lipoprotein (HDL) [8,20,21].

Attempts have been also undertaken to assess CD14 in periodontal tissues and in gingival crevicular fluid (GCF). McNamara et al. [22] found no differences in the levels of sCD14 in GCF between the CD14 monocyte receptor deficient patients and healthy subjects. Other authors, even though they observed no statistical differences between the levels of this molecule in GCF in P patients and healthy subjects, found certain correlation with clinical state. Namely, low sCD14 level in GCF was observed in patients with deeper periodontal pockets (>5 mm) and with their greater number [23]. Hayashi et al. [20] showed lack of gingival fibroblast staining by mCD14 antibodies. The authors cited above suggest that sCD14 play a basic role in the response to LPS present in periodontal pockets, and thus in bacteria-induced periodontal damage. Another authors have studied the in vivo espresssion profile and levels of mCD14 in healty and diseased gingival tissues. Clinically healty tissues showed greater levels of mCD14 than periodontal pocket tissues what may suggest that mCD14 is associated with favorable host responses to bacteria and may play role in maintaining periodontal homeostasis [24].

In conclusion basing on literature data it can be assumed that mCD14 and sCD14 play role in pathogenesis of aggressive periodontitis. However the relationship of these molecules with pathogenesis of periodontits is still unclear and needs further investigatons. According to our own results it can be taken into consideration that periodontal inflammation is a local disorder affecting a small fragment of the oral cavity and it seems likely that the chronic bacterial infection taking place there is not reflected in the peripheral parameters.

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