SECRETORY FUNCTION OF NEUTROPHILIC LEUKOCYTES OF THE PATIENTS WITH PERIODONTAL DISEASES

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INTRODUCTION

Severe periodontal disease has been reported to affect from 5 to 20% of the population (1). Periodontal diseases are the result of the interaction of the periodontal microflora and the multifaceted host response to the infection, aspects of this complex interaction are being identified and evaluated for both their static relationship to disease severity and their association with future disease progression (2). The host reaction to gingival microorganisms is characterized in part by an influx of neutrophilic leukocytes (NL), which is one of the most important steps in host defense (3, 4). NL establish the first defense barrier against the microbial invasion (3, 4, 5), establish the first defense barrier against the microbial invasion (3, 4, 5), they contain the necessary material for killing pathogenic microorganisms (11) and they are prone to phagocytosis, an increase in secretion of β-GD and LZ can be explained by overall increase in secretion of NL lysosomic enzymes, thus disclosing the mechanism of inflammatory periodontal tissue damage.

Lysozyme (LZ) is also found in crevicular fluid and saliva and acts against gram-positive and gram-negative bacteria (11). This enzyme acts on the beta-1, 4 glycosidic bonds of the peptido-glycans of bacterial cell walls and may be an important protective enzyme in periodontal diseases (12). Some studies have indicated a contradictory dependence between lysozyme levels and gingival inflammation (12). It is also found decreased concentrations of lysozyme in saliva of periodontitis patients but elevated levels in the crevicular fluid (11). Alkaline phosphatase (AP)-enzyme is enriched in the membranes of mineralizing tissue cells (osteoblasts) and is present in secretory function of NL affected by opsonized zymosane, non-opsonized E. coli was examined in 77 patients with gingivitis and periodontitis, and in 35 donors, free of internal diseases, by means of β-glucuronidase (β-GD), lysozyme (LZ). NL secreted higher levels of β-GD in incubation medium in patients with periodontitis (p≤0.001) subject to degree of periodontal lesion. NL affected by various antigens secreted higher levels of LZ into non-cellular matrix in patients with gingivitis and periodontitis comparing to control environment in analogous groups. Data obtained from this study suggest that in patients with periodontitis response of NL to bacterial stimuli is specific and subject to the degree of periodontal lesion. Our study showed a significant difference of AP activity in GCF subject to pocket depth and degree of periodontal lesion. Once NL are exposed to corpuscles prone to phagocytosis, an increase in secretion of β-GD and LZ can be explained by overall increase in secretion of NL lysosomic enzymes, thus disclosing the mechanism of inflammatory periodontal tissue damage.

Key words: periodontal diseases, alkaline phosphatase, β-glucuronidase, lysozyme.
NL granules (12). AP is produced by many cells within the periodontal environment, the principal sources being, polymorphonuclear leukocytes, bacterial sources within supra-and sub-gingival plaque, and osteoblast and fibroblast activity, with a small contribution from serum (14). Preliminary data for AP levels in supra-gingival plaque measure and demonstrate extremely low levels of AP (14). If the source of GCF AP is primarily the NL, through secondary granule release, it would form a potentially powerful marker of inflammation (14). The immune response of the macro-organism to oral microbial flora and its toxins is ambiguous (13, 15).

The aim of the present study was to determine the β-GD and LZ activity in incubation medium of NL and AP in gingival crevicular fluid (GCF) taken from patients with gingivitis and periodontitis and donors with no inflammation of the periodontium.

MATERIAL AND METHODS

Patients
Our study patients were selected from a large number of individuals with pathology of periodontal tissues who were examined clinically and radiographically and diagnosed as having gingivitis (gingival inflammation, bleeding detected during probing) or periodontitis (deep periodontal pockets, destruction of periodontal tissues surrounding the affected teeth and advanced bone loss). We included in our study only those patients with very marked signs of gingivitis and periodontitis using Russell’s (16) periodontal index (PI): from 0.0 to 8.0 points.

The study was performed on 112 systemically healthy subjects within an age range of 18 to 44 years: 35 donors with intact periodontium, they were rated PI from 0.0 to 0.2 points, 40 patients with gingivitis, was rated according to PI from 0.3 to 2.0 points, 37 patients with periodontitis, was rated according to PI from 4.0 to 8.0 points.

Secretory function of peripheral venous blood neutrophilic leukocytes
The secretory function of peripheral venous blood NL was determined accordingly to technique described by Talstad et al (17). Peripheral blood (5 ml) was taken from subjects who had abstained from morning meals by means of a sterile vacuum test-tube containing heparine (20 U/ml). Test-tubes were kept in a thermostat under an orifice the gingival crevice. By capillary action fluid entered the tube. The volume of fluid entering the tube can be precisely measured (19).

GCF was collected mesially and distally to each tooth after assessing the presence or absence of plaque, and registration of any other clinical parameters. The tooth was isolated with cotton rolls and dried with air.

Enzyme analysis
β-glucuronidase (β-GD) activity in incubation medium of NL was determined by J. Mead et al (20). Using 4-methylumbelliferyl-B-D-glucuronide as a substrate, and a spectrophotometer HITACHI MPF-2A (excitation wavelength -365 nm, emission wavelength -450 nm) was utilized for that purpose. Alkaline phosphatase (AP) activity was determined in gingival crevicular fluid by use of automatic biochemical analyzer „Monarch“ („Instrumentation Laboratory“). Lysozyme (LZ) was analysed using the spectrophotometric method (21), supplemented with Micrococcus luteus 2665.

Reagents: 4-methylumbelliferyl-B-D-glucuronide, 4-methylumbelliforene, Dulbecco buffer (pH 7.4), Hanks’ balanced salt solution (pH 7.3) were purchased from Sigma Chemical Co (USA).

Statistical analysis
Results were calculated and statistic analysis performed by means of SPSS MS for Windows. The comparison of quantitative values was performed using Student’s or Fisher’s F criterion. Continuous variables were described as Mean ± SEM. A probability value of <0.05 was taken as the level of statistical significant. The study design and completion followed ethical guidelines for conducting studies at Kaunas University of Medicine.

RESULTS

Data in Figure 1 shows that β-GD activity in incubation media (without affectors) of NL obtained from peripheral venous blood was similar in healthy subjects and patients with gingivitis and periodontitis (p>0.05), was equal to 0.99 ± 0.01 nM/ml/h, 1.080.12 nM/ml/h, 1.250.11 nM/ml/h. However, when incubation media were affected with opsonized zymosane, β-GD activity highly increased in both groups of patients (p<0.001) comparing to control environment, more markedly in patients with periodontitis, β-GD activity was dramatically higher in patients with periodontitis than in control group (p<0.001).

β-glucuronidase activity in incubation media of NL

Donors with intact periodontium n=35
Patients with gingivitis n=40
Patients with periodontitis n=37

* The were significant differences between donors with intact periodontium and patients with gingivitis and periodontitis in analagical incubation medium of NL (p<0.05)

Fig. 1. β-glucuronidase activity in incubation media of NL.
and even significantly higher that in patients with gingivitis (p<0.01). According to our data, β-GD activity highly increases (p<0.001) when incubation media of NL is affected with non-opsonized *E. coli* both in control group and in gingivitis and periodontitis groups comparing to response of NL in incubation media with phosphate buffer. Once affected with non-opsonized *E. coli* β-GD activity was significantly higher in patients with gingivitis (p<0.001) and periodontitis (p<0.001) comparing to donors with intact periodontal tissues.

LZ activity in incubation media of NL significant raised (Figure 2) affecting with opsonized zymosane of patients with gingivitis and periodontitis, as well as of donors with intact periodontium (p<0.001), comparing with control environments (phosphate buffer) in correspondent groups. LZ activity did not differ significantly in incubation media of NL of patients with gingivitis and periodontitis affected by opsonized zymosane (it was accordingly equal to 30,34.2 mg/L, 32,74.1 mg/L) in comparison with the enzyme activity in analogues incubation media of NL of donors with intact periodontium. Significantly increased LZ activity was not determined in incubation media of NL with non-opsonized *E. coli*, of patients with gingivitis and periodontitis, as well as of donors with intact periodontium (p>0.05), comparing with control environments (phosphate buffer). The increase in activity of NL secretion in non-cellular environment in patients with periodontitis was determined by exposing the incubation media to corpuscles prone to phagocytosis and bacterial toxins.

Data on AP activity in the samples of GCF given in Figure 3. According to our data AP activity in GCF patients with gingivitis and periodontitis was determined statistically significantly (p<0.001) higher as compared to donors with intact periodontal tissues.

**DISCUSSION**

As has been shown by investigations in recent years (22) periodontal disease is initiated by subgingival infection with selective Gram-negative bacteria, but the presence of microorganisms alone is not the only factor responsible for periodontal destruction (3, 23). The responses of the host to periodontopathic microorganisms are thought to be critically important (24).

NL are the principal cells of the host defense system and the primary protective cells against periodontal diseases (25). Released granule components from infiltrating leukocytes, such as lysosomal enzymes and reactive oxygen species, which are normally intended to degrade ingested microbes, can also lead to tissue destruction and amplification of the inflammatory response (26). It is well known that the activity of gram-negative bacteria present in dental plaque results in release of contents of lysosomal granules into non-cellular environment by NL with subsequent suppression of bacterial adhesion and growth (27, 28) as well as destruction of bacteria in non-cellular environment. Stimulating factors induce an increase in NL activity which stops microbial invasion and can even damage the tissues of macro organism (29, 30, 31). In localized aggressive periodontitis, in particular, uncontrolled neutrophil recruitment and activation has been demonstrated to lead to the aberrant release of an array of noxious agents intended to fight the bacteria, with the potential for causing further tissue damage (32). Neutrophilic leukocytes have been implicated to play a destructive role in the periodontal tissue breakdown process due to high levels of lysosomal enzymes, generation of superoxides and reactive oxygen derivatives (33). Our findings support data from other studies showing the relationship of increase in β-GD activity in sulcus fluid and periodontal tissue lesions (34, 35). Differences were determined between control group and the group of patients with gingivitis and periodontitis in terms of neutrophilic leukocyte degranulation according to β-GD activity induced by opsonized zymosane, non-opsonized *E. coli*. Non-opsonized *E. coli* and particularly opsonized zymosane significantly (p<0.001) induces β-GD activity that is dependent upon periodontal status. The highest activity was determined in patients with periodontitis and severe periodontal lesions, particularly when induced by opsonized zymosane. Lysozyme is found in crevicular fluid and saliva, and acts against gram-positive, and gram-negative bacteria (38), and is found decreased concentrations of lysozyme in saliva of periodontitis patients, but increase levels in the creicular fluid. No significant changes were observed neither in the crevicular fluid nor in the unstimulated or stimulated saliva (39). Our data show that LZ activity in incubation media of NL of patients with gingivitis and periodontitis was highly increased affecting with opsonized zymosane, comparing with control environments in correspondent groups. Recently Chapple et al (40) reported that monitoring AP activity in GCF permits prediction of periodontal attach-
ment loss. AP level in sulcular fluid notably increases in cases of untreated periodontitis (36, 37). Increased AP activity in teeth bearing orthodontic appliances might be due in part to gingival inflammation produced by plaque-receptive appliances, independently of clinically detectable dental displacements (44). Our data determined highly increased dependence of AP activity in GCF on lesions severity of periodontal tissue. Having determined the increase AP in GCF and β-GD, LZ activity in NL under the action of various antigens, a discussion can be raised on the activation of secretion of the entire system of lysosomal enzymes in these cells. According to Wilton (43), non-cellar release of lysosomal factors by NL from gingival crevicular exudate is possibly more efficient than phagocytosis. Subsequently, proteolytic enzymes can be incorporated into the explanation of mechanism of development of periodontal disease and give scientific grounds for oral hygiene in prevention of inflammatory periodontal disease.

REFERENCES


39. Chapple IIC, Garner I, Saxby MS, Moscrop H, Matthews JB. Prediction and diagnosis of attachment loss by enhanced chemilumini-