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What is This?
NEUTROPHIL FUNCTIONS IN PATIENTS WITH SEVERE PERIODONTAL DISEASE

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ABSTRACT

Recently several investigators have reported that neutrophil functions were depressed or defective in localized and generalized juvenile periodontitis. It has also been shown that patients with adult periodontitis exhibit no such abnormalities. Concerning rapidly progressing periodontitis, only a few reports have been provided. The purpose of the present study was to compare various neutrophil functions in patients with rapidly progressing periodontitis (RPP), generalized adult periodontitis (AP), and in periodontally healthy or control subjects.

Neutrophils and monocytes were obtained from heparinized peripheral blood of 13 RPP, 16 AP, and 18 control subjects. Cells were isolated by Percoll® discontinuous density gradient centrifugation. Neutrophil phagocytosis was examined by use of FITC-labeled bacteria, and the number of EA rosette-forming cells was determined. Random migration and chemotaxis of neutrophils and monocytes were evaluated with the Boyden chamber. In addition, opsonizing activity of plasma, effects of plasma on phagocytosis and chemotaxis, and lysosomal enzyme secretion were also determined.

Neutrophil phagocytosis was markedly depressed in RPP compared with AP and those with normal periodontal tissue. The number of phagocytizing neutrophils was decreased and seemed to be correlated with the number of EA rosette-forming cells. Random migration and chemotaxis of neutrophils and monocytes were not significantly different among these three study groups. No differences in other neutrophil functions could be found.

From these results, it was clear that neutrophil phagocytosis in RPP patients was depressed. These results suggest that neutrophil abnormalities may lead to decreased resistance to periodontal infections in adults with rapidly progressing forms of periodontal disease.

INTRODUCTION

Leukocytes have been implicated in host-parasite interactions, and previous studies have provided considerable support for the concept that the neutrophil plays an important protective role in the pathogenesis of periodontal diseases (Ciacciola et al., 1977; Genco and Mergenhagen, 1979; Genco et al., 1980; Van Dyke et al., 1982a; Genco and Slots, 1984; Miller et al., 1984).

Since Ciacciola et al. (1977) demonstrated that neutrophil phagocytosis and chemotaxis were defective in patients with localized juvenile periodontitis (LJP), numerous investigators have reported that neutrophil functions were depressed or defective in certain types of periodontal diseases (Altman et al., 1982; Ellegaard et al., 1984; Lavine et al., 1979; Van Dyke et al., 1982b; Van Dyke, 1985; Vandesteen et al., 1984; Manouchehr-Pour et al., 1981; Cogen et al., 1983; see review in Genco and Slots, 1984).

While neutrophil dysfunction in LJP has been well-known, only a few reports have appeared concerning rapidly progressive periodontitis (RPP) (Altman et al., 1982, 1985; Page et al., 1985). Altman et al. (1985) have demonstrated defective chemotaxis of neutrophils and monocytes in some patients with RPP compared with those of periodontally healthy subjects. Page et al. (1985) have also shown suppressed chemotaxis of both cells at a certain concentration range of N-formylmethionyl-leucyl-phenylalanine (FMLP). In addition, enhanced random migration has been seen in neutrophils and monocytes of RPP patients (Page et al., 1985).
Thus, abnormalities of leukocyte motility in some patients with RPP have been shown. On the other hand, it has been reported that adult periodontitis (AP) patients do not exhibit these abnormalities of neutrophil functions (Van Dyke et al., 1980; Altman et al., 1984; Genco and Slots, 1984; Van Dyke et al., 1980).

The status of other neutrophil functions—such as phagocytosis, intracellular killing, and lysosomal enzyme secretion—in RPP is still unclear, although those functions also play important roles in host defense.

The purpose of the present study was to survey and to compare various neutrophil functions in patients with RPP, AP, and control subjects. Neutrophil phagocytosis, neutrophil and monocyte chemotaxis, and lysosomal enzyme secretion from neutrophils of these groups were determined. Particularly, detailed studies on phagocytosis were attempted.

**MATERIALS AND METHODS**

**Neutrophils and Monocytes**

Based on historical, clinical, and radiographic findings, patients were assigned a diagnosis of RPP or AP, generally according to previously described criteria (Page and Schroeder, 1982; Page et al., 1983). Neutrophils and monocytes were isolated according to the method of Hjorth et al. (1981). Briefly, cells were separated from heparinized peripheral blood of 13 RPP, 16 AP, and 18 controls by Percoll® (Pharmacia Fine Chemicals, Uppsala, Sweden) discontinuous density gradient centrifugation. Neutrophils and monocytes were suspended in Gey's balanced salt solution containing 2% bovine serum albumin at densities of $2.5 \times 10^6$ cells/mL and $3.0 \times 10^6$ cells/mL, respectively. Viability of these cells was determined to be more than 95% by the trypan blue dye exclusion test. Neutrophil populations were greater than 90% pure, as determined by Giemsa stain, and monocytes comprised about 15% of mononuclear cell populations, as revealed by alpha-naphthyl acetate esterase stain.

**Neutrophil Phagocytosis**

Neutrophil phagocytosis was assayed by the method of Hed (1977). Heat-inactivated *Staphylococcus aureus* (ATCC 6538) was labeled with fluorescein isothiocyanate (FITC, Sigma Chemical Company, St. Louis, MO) and opsonized with 10-fold-diluted type AB serum. Bacteria were suspended in PBS at a density of $1.0 \times 10^9$ cells/mL. A 900-μL aliquot of the neutrophil suspension and 100 μL of the bacterial suspension were mixed and incubated at 37°C for 15 min (neutrophil : bacteria = 1:40). After addition of 0.2 mg/mL of crystal violet to the reaction mixture, neutrophil phagocytosis was determined by the enumeration of attached and internalized bacteria with the fluorophotomicroscope ($\times 1000$).

**EA Rosette Formation of Neutrophils**

EA rosette formation of neutrophils was determined according to the method of Nagumo et al. (1982), utilizing trypsin-treated sheep red blood cells (SRBC) and rabbit anti-SRBC IgG (Cappel Laboratories, Inc., Cochranville, PA). Neutrophils and EA (1 : 400) were mixed and incubated at room temperature for 60 min. Neutrophils which attached three or more EA were counted as EA rosette-forming cells.

**Neutrophil and Monocyte Chemotaxis**

Neutrophil and monocyte chemotaxis were determined with a modified Boyden chamber as previously described (Snyderman et al., 1968). Neutrophil suspensions were placed in the upper of two compartments of the chamber, separated by a nitrocellulose micropore filter (5-μm pore size). The lower compartment contained Gey's gelatin veronal buffer (GVB) with various concentrations of N-formyl-methionyl-leucyl-phenylalanine (FMLP, Protein Research Foundation, Minno, Osaka, Japan) as chemo-attractant. After incubation at 37°C for 60 min in 5% CO₂, the filters were fixed and stained by Weigert's hematoxylin solution. Neutrophil chemotaxis was determined by the counting of the number of migrating cells on the distal surface of the filter. Monocyte chemotaxis was assayed similarly, except 8-μm-pore-size filters were employed, endotoxin-activated serum (EAS) was used 30-fold-diluted with Gey's GVB as a chemo-attractant, and incubation was for 120 min.

**Statistical Analysis**

All experimental data from the three study groups were analyzed with the Student *t* test.

**RESULTS**

Neutrophil phagocytosis in RPP patients was significantly depressed compared with AP and control groups. Figs. 1(A) and (B) show the results of measurements of attached and internalized bacteria, respectively, in the process of phagocytosis by neutrophils. The mean (± S.E.) numbers of attached bacteria per 100 neutrophils in the three groups—control, RPP, and AP—were 104.7 ± 5.3, 71.2 ± 9.6, and 117.7 ± 6.8, respectively. There was a significant difference between control and RPP (*p*<0.01); however, no difference was found between control and AP. In the case of internalized bacteria, similar results were obtained. The mean numbers of internalized bacteria per 100 neutrophils in the three groups were 243.7 ± 11.5, 151.1 ± 16.8, and 241.5 ± 10.5, respectively. A significant difference was found only between control and RPP (*p*<0.001).

EA rosette formation by neutrophils is demonstrated in Fig. 2. The mean numbers of EA rosette-forming cells in the three groups—control, RPP, and AP—were 72.2 ± 2.8, 48.1 ± 2.7, and 71.6 ± 1.6 per
Fig. 1—Phagocytic activity of neutrophils from 18 control subjects (●), 13 RPP patients (○), and 13 AP patients (□). (A) Attachment phase; values are expressed as the number of bacteria attached to 100 neutrophils. (B) Internalization phase; values are expressed as the number of bacteria internalized by 100 neutrophils.

100 neutrophils, respectively. There was a significant difference (p<0.01) between control and RPP. The relationship between internalizing neutrophils and EA rosette-forming neutrophils is given in Fig. 3. The number of internalizing neutrophils increased with an increase in the number of EA rosette-forming neutrophils, and these two parameters were well-correlated (r = 0.627, p<0.01).

The results of neutrophil chemotactic responses to various concentrations of FMLP are shown in Fig. 4. Random migration and chemotaxis were found to vary individually; however, they were found not to be different at this concentration range among the three study groups. Although data are not shown, the monocyte chemotactic response to EAS was also not different.

Other functions tested revealed only similarities among the three groups. In brief, opsonizing activity of plasma was determined by neutrophil phagocytosis using bacteria opsonized with plasma instead of AB serum. The effects of plasma on neutrophil phagocytosis and chemotaxis were measured by use of neutrophils pre-incubated with 10-fold-diluted plasma for various periods. Extracellular release of the lysosomal enzyme, lysozyme, from neutrophils was measured by a turbidimetric method after stimulation with cytochalasin B. The results of these experiments showed no differences among these groups (data not shown).

DISCUSSION

Various neutrophil functions of patients with RPP, AP, and control subjects were surveyed and com-

Fig. 2—EA rosette formation by neutrophils from eight control subjects (●), seven RPP patients (○), and five AP patients (□). Values are expressed as the number of EA rosette-forming neutrophils per 100 neutrophils counted.

Fig. 3—The correlation between EA rosette formation and internalization of neutrophils. The horizontal axis shows the number of internalizing cells per 100 neutrophils; the vertical axis shows the number of EA rosette-forming cells per 100 neutrophils (r is the correlation coefficient).
The most important thing may be the individuality of patients. Another reason for these differences may reflect methodological differences in assessment of leukocyte motility. The most important thing may be the difficulty in clearly defining patients described as RPP without accurate measurements of disease at several intervals.

Lysosomal enzyme secretion from neutrophils did not differ among the RPP, AP, and control groups, as was seen for chemotaxis. This may be reasonable to expect, because the FMLP receptor on the neutrophil is involved in both responses (Gallin et al., 1978).

The present study suggests that depression of neutrophil phagocytosis is one of the distinguishing features of RPP and might contribute to the rapid progression of this disease. The assessment of this function may be important and useful for explaining various types of periodontal pathologies.

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