Neutrophil Dysfunctions, IL-8, and Soluble L-Selectin Plasma Levels in Rapidly Progressive Versus Adult and Localized Juvenile Periodontitis: Variations According to Disease Severity and Microbial Flora

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Neutrophil Dysfunctions, IL-8, and Soluble L-Selectin Plasma Levels in Rapidly Progressive Versus Adult and Localized Juvenile Periodontitis: Variations According to Disease Severity and Microbial Flora

Jean Gavit,† Pham M. C. Dang,* Sylvie Chollet-Martin,* Monique Brion,† Michel Sixou,‡ Jacques Hakim,* Marie-Anne Gougerot-Pocidalo,1* and Carole Elbim*

We used flow cytometry to analyze the expression of adhesion molecules and the oxidative burst of whole-blood polymorphonuclear neutrophils (PMN) from 26 patients with periodontitis. Three different clinical entities were studied: adult periodontitis (AP), localized juvenile periodontitis (LJP), and rapidly progressive periodontitis (RPP). Unstimulated PMN from the patients showed reduced Lewis x, sialyl-Lewis x, and L-selectin expression relative to those from healthy control subjects. These alterations were present whatever the severity of periodontal disease. However, PMN from RPP patients showed increased basal H2 O2 production and decreased L-selectin shedding. These latter impairments, which correlated with increased IL-8 plasma levels, could contribute to initial vascular damage. In addition, decreased IL-8 priming of H2 O2 production by PMN from RPP patients could account for a lower bactericidal capacity of PMN, leading to the large number of bacteria in the subgingival region of RPP patients. Soluble L-selectin plasma levels were also decreased in the RPP group, indicating more severe or diffuse endothelial damage. These abnormalities were not found in the patients with less destructive forms of periodontitis (AP and LJP). Porphyromonas gingivalis, a bacterial pathogen known to increase IL-8 production by PMN, was found in the periodontal pockets of RPP patients only. These results show links among PMN abnormalities, the clinical form of periodontitis, and the gingival bacterial flora. The Journal of Immunology, 1999, 163: 5013–5019.

Periodontal diseases, particularly adult periodontitis (AP)2 and early-onset periodontitis (EOP), i.e., localized juvenile periodontitis (LJP) and rapidly progressive periodontitis (RPP), have very different clinical features that correspond to various degree of periodontal destruction. RPP is a rapidly destructive form of periodontitis (1), affecting most all of the teeth and typically emerging in the late second or third decade of life. Severe florid gingival inflammation and rapid loss of attachment are common, frequently culminating in tooth loss. AP starts in early adulthood and progresses slowly but with unpredictable episodes of activity and quiescence (2, 3). Clinical manifestations usually occur in the third or fourth decade of life. The number and pattern of affected teeth vary widely among individuals. It is likely that a consortium of bacteria cause AP. LJP usually starts between the ages of 14 and 17 and mainly affects the incisors and first molars. There is little bacterial plaque accumulation and gingival inflammation, but severe angular bone loss occurs rapidly around the affected teeth (4). The mechanisms underlying these clinical differences between the different forms of periodontitis are poorly understood.

Polymorphonuclear neutrophils (PMN) are the most abundant immune cells in inflammatory gingival sites of patients with periodontitis, and their pathogenic role in this setting has been suggested (5–7). One of the major steps leading to blood PMN migration to an inflammatory site is the modulation of adhesion molecule expression on both PMN and endothelial cells. In particular, proinflammatory cytokine-induced shedding of the L-selectin (CD62L), followed by increased expression of the β2 integrin CD11b/CD18 at the PMN surface, is one of the main mechanisms underlying transendothelial migration (8, 9). The Lewis x (Le x) and its sialylated derivative, sialyl-Lewis x (sLe x), also participate in the complex process of adhesion between leukocytes and endothelial cells, in particular involving the selectin adhesion molecules (10, 11). Altered expression of these adhesion molecules could thus influence PMN migration and lead to inappropriate release of reactive oxygen species, thereby initiating tissue injury (12, 13). In parallel, plasma levels of soluble L-selectin (sL-selectin) have been reported to reflect cell-endothelial interactions and endothelial damage (14, 15). PMN dysfunctions leading to inappropriate tissue destruction may thus play a key role in the pathogenesis of periodontal diseases and may be involved in disease severity. We recently found marked PMN dysfunctions in RPP, related to increased IL-8 production (16). Contradictory results on both PMN functions (chemotaxis, phagocytosis, and oxidative burst) and surface receptor expression (e.g., CD11b/CD18) have been reported in AP and LJP. Several authors have found that
PMN from periodontitis patients have normal or even enhanced responses (17–20), whereas others have found that both resting and stimulated PMN have diminished activities (21, 22). These discrepancies could be due to the use of PMN from patients at different stages of periodontal disease, and to the fact that most studies have involved PMN isolated from their blood environment.

In this study, PMN abnormalities were analyzed in fresh whole blood to minimize procedure-related changes in surface receptor expression (23). Flow cytometry, which can be used to study events at the single-cell level, was chosen to analyze adhesion molecule expression at the PMN surface, especially L-selectin, CD11b/CD18, Lea, and sLea Ags, and also H2O2 production by PMN. In parallel, we determined circulating levels of sL-selectin and proinflammatory cytokines. We also analyzed the bacterial population in periodontal pockets to identify a possible link between the gingival microbial flora, PMN dysfunctions, and the clinical form of periodontitis.

Materials and Methods

**Patients**

Ten patients (four women and six men; mean age, 39 ± 4 years) with RPP, eight patients with AP (three women and five men; mean age, 55 ± 2 years), and eight patients with LJP (four women and four men; mean age, 19 ± 3 years) were referred to A. Chenevier Hospital ( Créteil, France). Controls from medical and dental histories were recorded, and no systemic diseases potentially altering neutrophil functions were identified. None of the patients had taken antibiotics or corticosteroids during the previous 6 mo. The diagnosis of periodontitis was based on clinical examination and dental radiography, using published criteria (24). After informed consent had been obtained, blood was collected in sterile lithium heparinate- or EDTA-treated tubes, placed on ice, and analyzed immediately. Fourteen members of the laboratory staff served as controls.

**Reagents**

The reagents were as follows: human rTNF-α and human recombinant endothelial cell-derived IL-8 (Genzyme, Cambridge, MA); 2,7′-dichlorofluorescein-diacetate (DCFH-DA; Eastman Kodak, Rochester, NY); fMLP and edetate (LPS) from *Escherichia coli* (055 = B5) (Sigma, St. Louis, MO); R-PE-conjugated monoclonal mouse anti-human CD11b Ab and FITC-conjugated monoclonal mouse anti-human Lea (CD15; Dako-patts, Glostrup, Denmark); FITC-conjugated monoclonal mouse anti-human CD18 Ab and monoclonal mouse Ab to human sLea (Becton Dickin-son Immunocytometry Systems, San Jose, CA); monoclonal mouse Ab to human L-selectin (CD62L, Coulter Immunology, Hialeah, FL); and FITC-conjugated goat anti-mouse (GAM) Ab (Nordic Immunology, Tilburg, The Netherlands).

Stock solutions of DCFH-DA (50 mM) and fMLP (10−7 M) were prepared in DMSO and stored at −20°C. Lyophilized IL-8 was dissolved in PBS containing 0.1% sterile human serum albumin (PBS; Pharmacia Fine Chemicals, Uppsala, Sweden) and stored at −80°C. The different solutions were diluted in PBS just before use. All the cytokines used in this study were certified by the manufacturer to contain <0.1 ng of LPS per milligram of protein.

**Cytokine and sL-selectin assays**

Blood was collected into sterile EDTA-treated vacuum tubes, transported on ice to the laboratory, and immediately centrifuged at 1500 × g for 15 min at 4°C to avoid cytokine synthesis or breakdown in vitro. Plasma samples were stored at −70°C for no longer than 15 days before assay. Cytokines in plasma were assayed in duplicate using immunoenzymatic assays for IL-6, IL-8, IL-10, and TNF-α, all with a detection limit of 15 pg/ml (Medigenix, Brussels, Belgium, for IL-6 and IL-10; R&D Systems, Abingdon, U.K., for IL-8; and Immunotech, Marseille, France, for TNF-α). The assays were standardized by comparing the results to those of standards from the National Institute for Biological Standards and Control (England). Cytokine recovery was 102 ± 4% from normal plasma spiked with recombinant human cytokines. sL-selectin was assayed by using an immunoassay (Immunotech) with a detection limit of 15 ng/ml.

**Determination of adhesion molecule expression at the PMN surface**

Whole-blood samples were either kept on ice or incubated with fMLP (10−6 M) or PBS at 37°C for 5 min. To study CD11b, CD18, and CD15 (Lea) expression, samples (100 μl) from each patient were then incubated with mAbs (PE-anti-CD11b, FITC-anti-CD18, FITC-anti-CD15) for 30 min at 4°C. To study L-selectin and sLea Ag expression, samples were incubated with nonconjugated anti-CD62L and anti-sLea Abs for 30 min at 4°C, washed with ice-cold PBS, and then incubated at 4°C for 30 min with FITC-goat anti-mouse Ab. Red cells were lysed with FACS lysing solution. After one wash with ice-cold PBS, the cells were resuspended in 1% paraformaldehyde-PBS and kept on ice until flow cytometry. Nonspecific Ab binding was determined on cells incubated with the same concentration of an irrelevant Ab of the same isotype.

**H2O2 production**

H2O2 growth was measured by using a flow cytometric assay derived from the technique described by Bass et al. (25) and others (26, 27). One milliliter of fresh blood collected onto preservative-free lithium heparinate (10 U/ml) was preincubated for 15 min with 2′,7′-DCFH-DA (100 μM) in a water bath at 37°C with gentle horizontal agitation. DCFH-DA diffused into the cells and is hydrolyzed into 2′,7′-dichlorofluorescein (DCFH); during the PMN oxidative burst, non fluorescent intracellular DCFH is oxidized to highly fluorescent dichlorofluorescein (DCF) by H2O2 in the presence of peroxidase. The samples were then incubated with TNF-α (10 U/ml), LPS (5 μg/ml), or IL-8 (50 ng/ml) diluted in PBS, or with PBS alone, at 37°C for 30 min. FMLP diluted in PBS (10−8 M final concentration) or PBS was added for 5 min at 37°C. The reaction was stopped and red cells were lysed with FACS lysing solution (Becton Dickinson Immunocytometry Systems). After one wash (400 × g for 5 min), white cells were suspended in 1% paraformaldehyde-PBS. The fixed samples were kept on ice until flow cytometric analysis on the same day. As previously reported, the DCFs lysing solution modified neither the amount of DCF generated nor the expression of activation markers such as CR3, as shown by flow cytometry (26). Moreover, PMN viability was not altered in our conditions, as assessed in terms of propidium iodide exclusion by means of flow cytometry. Finally, we checked that DCF did not diffuse out of the cells (data not shown).

**Flow cytometry**

We used a Becton Dickinson FACScan with a 15-mW, 488-nm argon laser. PMN functions were analyzed using Lysis II software. Forward and side scatter were used to identify the granulocyte population and to gate out other cells and debris. The purity of the gated cells was assessed by using FITC- or PE-conjugated CD3, CD45, CD14, and CD15 Abs (Becton Dick-inson). Five thousand events were counted per sample, and the fluorescences were amplified by 4-decade logarithmic amplifiers. The green fluorescence of DCF and FITC-Abs was recorded from 515 to 545 nm; the red fluorescence of PE-anti CD11b was recorded from 563 to 607 nm. In all cases, unstained cells were used and the photomultiplier settings were adjusted so that the unstained cell population appeared in the lower left-hand corner of the fluorescence display. In the dual-color analysis, single-cell controls were used to optimize signal compensation. All the results were obtained with a constant photomultiplier gain. Mean fluorescence intensity (MFI) was used to quantify the cell responses.

**Microflora analysis**

The subgingival microflora was analyzed in the three patient groups. The sites were isolated from the saliva with cotton rolls. The sampling method chosen was to use sterile absorbent paper (Johnson and Johnson, New Brunswick, NJ), which efficiently collects anaerobic bacteria (28). The pa-er points were inserted into the gingival sulcus until resistance was felt and were left in place for 10 s. Each paper point was then placed in a tube containing 2 ml of semisolid anaerobic transport medium, VGMA III. The samples were then treated within 24 h. The bacteria were dispersed with a vortex mixer at the maximal setting for 60 s in an anaerobic chamber. The nonselective medium was a blood agar base supplemented with various growth factors. Colonies of pathogens were identified by atmospheric growth characteristics, Gram staining characteristics and biochemical (API 20A) and enzymatic tests (rapid ID 32A strips). Following incubation, the total number of CFU/ml of plaque suspension of each sample were calculated from Geplates (Polylabo, Strasbourg, France). CFU/ml values for
Correlations were identified by means of the Spearman correlation coefficient of all the pathogens in the different groups of patients.

Counts of less than 10³ CFU/ml were considered negative. Percentages of recovery were calculated from the total CFU/ml. Bacterial species that were considered significant were Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Fusobacterium nucleatum, Eikenella corrodens, Capnocytophaga ochracea, Campylobacter rectus, Bacteroides forsythus, and Wolinella recta.

Three groups of patients. In particular, neutrophil counts were normal in the patients, suggesting the absence of increased granulopoiesis. Moreover, the hemocytometer (Bayer H1) did not detect the presence of immature PMN in the blood of any patients studied.

Table I. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Range</th>
<th>RPP² (n = 10)</th>
<th>LJP³ (n = 8)</th>
<th>AP⁴ (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocyte count&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,000–10,000</td>
<td>6,753 ± 476</td>
<td>6,095 ± 687</td>
<td>7,275 ± 657</td>
</tr>
<tr>
<td>% neutrophils</td>
<td>40–75</td>
<td>61 ± 2.4</td>
<td>55.4 ± 3.9</td>
<td>56.6 ± 1.5</td>
</tr>
<tr>
<td>Neutrophils count&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,500–7,500</td>
<td>4,254 ± 356</td>
<td>3,435 ± 562</td>
<td>4,125 ± 421</td>
</tr>
<tr>
<td>% eosinophils</td>
<td>1–5</td>
<td>2.2 ± 0.5</td>
<td>3.2 ± 0.9</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Eosinophils count&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50–300</td>
<td>158 ± 44</td>
<td>202 ± 69</td>
<td>143 ± 36</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are means ± SEM.
<sup>b</sup> Values per microliter.

Table II. L-selectin, CD11b, Le<sup>a</sup>, and sLe<sup>x</sup> Ag expression by resting PMNs from controls and patients with RPP, LJP, and PA<sup>a</sup>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>MFI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-selectin</td>
<td>CD11b</td>
</tr>
<tr>
<td>Controls (n = 14)</td>
<td>491 ± 32</td>
<td>136 ± 5</td>
</tr>
<tr>
<td>RPP (n = 10)</td>
<td>378 ± 37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>172 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LJP (n = 8)</td>
<td>296 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145 ± 3</td>
</tr>
<tr>
<td>AP (n = 8)</td>
<td>291 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Whole blood maintained at 4°C was incubated with anti-CD62L, anti-CD11b, anti-Le<sup>a</sup>, and anti-sLe<sup>x</sup> Abs for 30 min. MFI was recorded as described in Materials and Methods. Values obtained with an irrelevant Ab of the same isotype were subtracted. Values are given as means ± SEM.
<sup>b</sup> Significantly different from control values (p < 0.05).

Table III. L-selectin, Le<sup>a</sup>, CD11b, and sLe<sup>x</sup> Ag expression by fMLP-stimulated PMNs from controls and patients with RPP, LJP, and PA<sup>a</sup>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>MFI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-selectin</td>
<td>CD11b</td>
</tr>
<tr>
<td>Controls (n = 14)</td>
<td>44 ± 6</td>
<td>2081 ± 122</td>
</tr>
<tr>
<td>RPP (n = 10)</td>
<td>167 ± 42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1714 ± 230</td>
</tr>
<tr>
<td>LJP (n = 8)</td>
<td>39 ± 3</td>
<td>2184 ± 59</td>
</tr>
<tr>
<td>AP (n = 8)</td>
<td>32 ± 4</td>
<td>2049 ± 98</td>
</tr>
</tbody>
</table>

<sup>a</sup> After incubation with fMLP at 10⁻⁶ M for 5 min at 37°C, whole-blood samples were incubated with anti-CD62L, anti-CD11b, anti-Le<sup>a</sup>, and anti-sLe<sup>x</sup> Abs for 30 min. MFI was recorded as described in Materials and Methods. Values are given as means ± SEM.
<sup>b</sup> Significantly different from control values (p < 0.05).
<sup>c</sup> Significantly different from control values (p < 0.05).

**Expression of adhesion molecules at the PMN surface**

As shown in Table II, the MFI of anti-L-selectin Ab binding to unstimulated PMN was significantly lower in all the groups of patients than in the healthy control subjects. No significant difference was observed among the three groups of patients. Incubation with PBS alone did not significantly modify values relative to resting PMN maintained at 4°C (data not shown). After stimulation with fMLP (Table III), L-selectin was no longer detectable on PMN from control subjects or AP and LJP patients. In fact, the MFI obtained with the anti-LAM Ab was always lower than the MFI obtained with an irrelevant Ab of the same isotype. In contrast, L-selectin was still detectable after fMLP stimulation on PMN from RPP patients.

Lower Le<sup>a</sup> and sLe<sup>x</sup> Ag expression at the surface of resting and stimulated PMN was observed in the three groups of patients than in the healthy control subjects (Tables II and III). CD11b expression on resting PMN was significantly increased in RPP and AP than in controls (Table II). After fMLP stimulation, CD11b expression did not differ significantly between the controls and all groups of patients (Table III). Similar results were obtained concerning CD18 expression at the PMN surface (data not shown).

**Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Fusobacterium nucleatum, Eikenella corrodens, Capnocytophaga ochracea, Campylobacter rectus, Bacteroides forsythus; and Wolinella recta** were obtained from selective plates, and their relative percentages of recovery were calculated from the total CFU/ml. Bacterial counts of less than 10³ CFU/ml were considered negative.

**Statistical analysis**

Results are expressed as means ± SEM. The group means were compared by using ANOVA followed by a multiple comparison of means with Fisher’s least-significant-difference procedure; p values of 0.05 or less were considered significant. Correlations were identified by means of the Spearman rank correlation coefficient (ρ). χ² analysis was used to compare the distribution of all the pathogens in the different groups of patients.

**Results**

**Characteristics of blood leukocytes in the patients groups**

As shown in Table I, the absolute leukocyte counts and the differential neutrophils and eosinophils counts in all the patients groups were within the normal range and did not differ among the three groups of patients. In particular, neutrophil counts were normal in the patients, suggesting the absence of increased granulopoiesis. Moreover, the hemocytometer (Bayer H1) did not detect the presence of immature PMN in the blood of any patients studied.
followed by stimulation with 10-6 M fMLP for 5 min at 37°C for 30 min, and then with PBS or fMLP at 10-6 M for 5 min. MFI of dichlorofluorescein (DCF) was recorded as described in Materials and Methods. Values are given as means ± SEM.

* Significantly different from controls, AP, and LJP values (p < 0.05).

H₂O₂ production by PMN in whole blood
As shown in Table IV, H₂O₂ production was significantly higher in unstimulated PMN from RPP patients than from healthy controls, the latter expressing low background fluorescence. H₂O₂ production by unstimulated PMN from LJP and AP patients was similar to that of healthy controls and significantly lower than in production by unstimulated PMN from LJP and AP patients. In particular, H₂O₂ production by PMN from RPP patients thus showed an increased basal level of H₂O₂ production, which was not further enhanced after priming by IL-8, whereas PMN from the two other groups of patients (LJP and AP) behaved like those from the healthy controls in terms of H₂O₂ production. In contrast, after TNF and LPS priming followed by fMLP stimulation, H₂O₂ production by PMN from the three groups of patients did not significantly differ from those of healthy controls (Table V), which values were in accordance with previous data (26).

Cytokine and sL-selectin plasma levels
As shown in Table VI, IL-8 plasma levels were significantly higher in RPP patients than in the other patients and the controls, in whom IL-8 was undetectable. In contrast, IL-6, TNF-α, and IL-10 plasma levels were not significantly different in the patient groups and controls, values always being below 15 pg/ml. Moreover, after fMLP stimulation IL-8 plasma levels correlated positively with L-selectin expression on the PMN surface (p = 0.7, p = 0.0001), as well as spontaneous H₂O₂ production by PMN (p = 0.4, p =
and correlated negatively with H$_2$O$_2$ production after IL-8 priming ($r$ = 0.6, $p$ = 0.0002). As shown in Table VI, sL-selectin plasma levels were significantly lower in the RPP group than in healthy subjects and LJP/PA groups.

Subgingival microbial population

*P. gingivalis* was present in all the RPP patients, whereas it was undetectable in the AP and LJP patients (Table VIII). In addition, *P. gingivalis* was abundant, ranging in numbers from $10^5$ to $5 \times 10^7$ CFU/ml. The $\chi^2$ test showed that *Actinobacillus actinomycetemcomitans* was significantly more frequent in LJP patients than in RPP and AP patients ($p < 0.0005$), in agreement with the literature (29, 30). $\chi^2$ testing of the distribution of the other pathogens (*Prevotella intermedia, Fusobacterium nucleatum, Eikenella corrodens,* and *Campylobacter rectus*) showed no significant difference among the three groups of patients. Nevertheless, the mean total number of pathogens excepting *P. gingivalis* was clearly higher in patients with RPP ($1.7 \times 10^7$ CFU/ml) than in those with AP ($1.6 \times 10^6$ CFU/ml) and LJP ($1 \times 10^6$ CFU/ml).

Effect of professional treatment

All RPP patients were treated by means of hygiene motivation, scaling, root planing, and surgery. This led to almost complete disappearance of clinical gingival inflammation. Five patients were again studied following therapy. Normal basal H$_2$O$_2$ production (MFI = 33.8 ± 5.2) and normal H$_2$O$_2$ production after IL-8 priming corresponding to control values (MFI = 74.8 ± 5.3) were observed. Values, before and after treatment in each patient, are represented in Fig. 1. After treatment, IL-8 plasma levels were undetectable (<15 pg/ml).

Discussion

Our results show that resting PMN from patients with RPP, LJP, and AP express reduced levels of Le$^x$, sLe$^x$, and L-selectin at their surface. In addition, RPP patients showed a decreased shedding of L-selectin after fMLP stimulation, an increased basal production of H$_2$O$_2$ by PMN, a diminished PMN oxidative burst in response to formyl peptide after ex vivo priming with IL-8, and increased IL-8 plasma levels, whereas these abnormalities were not found in the two less severe forms of periodontitis (AP and LJP). Furthermore, *P. gingivalis*, a bacterial pathogen able to increase IL-8 production by PMN, was only found in periodontal pockets from RPP patients. Finally, sL-selectin plasma levels were only decreased in the RPP group, suggesting binding to activated endothelial cells.

PMN transendothelial migration involves a series of precise events that are regulated by constitutive and inducible adhesion molecules (31). The selectin family of adhesion molecules on both PMN (L-selectin) and endothelial cells (E- and P-selectin) are involved in the initial interaction of circulating PMN with the endothelium, in a process known as “rolling adhesion” (9). At the PMN surface, the specific carbohydrate ligands recognized by endothelial selectins are Lex Ag (CD15) and its sialylated derivate (sLe$^x$), which are expressed on glycolipids and on several glycoproteins (32). In response to stimuli such as endothelium-derived cytokines (e.g., IL-8), L-selectin is rapidly shed by proteolytic cleavage from the PMN surface, and a $\beta_2$ integrin-dependent phase of firm adhesion then occurs (8).

There have been conflicting reports on the expression of CD11b/CD18 and L-selectin at the surface of PMN from patients with
periodontitis (17, 19, 21, 22, 33, 34). The apparent discrepancies could at least in part be due to methodological differences. In particular, PMN isolation procedures have been shown to modify the surface expression of molecules that are not detectable in whole blood and that may be markers of PMN activation (23). Activation due to isolation procedures used in other reports might thus have masked differences between patients and healthy controls. We clearly observed decreased expression of L-selectin at the surface of resting PMN from all the patients as compared with the control subjects. Diminished L-selectin expression related to a shedding process has been reported to occur during PMN activation (9). The decreased L-selectin expression was associated with reduced expression of Leα and sLeα Ags on PMN. As the Leα and sLeα structures are carried by different glycoproteins (32), and especially L-selectin (35, 36), the decreased Leα and sLeα expression could also partly be explained by decreased L-selectin expression. This altered basal expression of adhesion molecules at the PMN surface in the three groups of patients is in keeping with PMN activation, probably related to the subgingival bacterial invasion present in the three groups of patients, although in a qualitatively and quantitatively different manner. In addition, we found slightly increased basal expression of another activation parameter, the β2 integrin CD11b, in RPP and AP patients.

After stimulation with fMLP, L-selectin expression was no longer detectable on PMN from control subjects or from patients with AP and LJP. In contrast, L-selectin was still detectable after fMLP stimulation on PMN from RPP patients, indicating more profound L-selectin dysregulation in RPP patients. In contrast, we observed that stimulated PMN from all three groups of periodontitis patients showed normal maximal CD11b surface expression. Similar decreased shedding of L-selectin, associated with a normal maximal expression of CD11b, has been reported in patients with diseases other than periodontitis (37, 38). This dysregulated L-selectin expression that we found in RPP patients could immobilize PMN on the endothelium and lead to initial vascular damage (39). Furthermore, we found low levels of sL-selectin in plasma from RPP patients. Decreased sL-selectin levels may reflect sequestration of sL-selectin by widespread binding to activated endothelium in microvascular beds. This is supported by immunohistochemical findings showing that sL-selectin specifically binds to luminal surfaces of high endothelial venules at sites of inflammation (40). Thus, low levels of sL-selectin in RPP patients probably reflect diffuse activation of the endothelium.

Activation of PMN at inflammatory sites leads to the production of reactive oxygen species in a phenomenon known as the oxidative burst. This plays a key role in defenses against bacterial and fungal infections and may also potentiate inflammatory reactions, leading to tissue injury. We found an increase in H2O2 production by whole-blood unstimulated PMN from RPP patients relative to healthy control subjects. In contrast, basal H2O2 production was normal in AP and LJP patients. This increased H2O2 production could be related to PMN activation by P. gingivalis, which predominated in periodontal lesions of patients with RPP and was absent from gingival samples of AP and LJP patients. LPS from P. gingivalis has been reported to strongly prime H2O2 production by PMN in response to fMLP (41). Moreover, P. gingivalis actively invades endothelial cells (42) and increases production of IL-8 (43), which has been reported to activate PMN (44). Mikolajczyk-Pawlinska et al. (45) demonstrated that soluble gingipains, the major cysteine proteinases synthesized by P. gingivalis, can convert IL-8 to a more potent species truncated at the amino-terminus, followed by slower degradation of chemokine biological activity. Even though various bacteria were found in the three groups of patients, the number of pathogens was about 10-fold higher in RPP patients than in the LJP/AP patient groups. In RPP patients, P. gingivalis periodontal invasion could thus increase endothelial IL-8 production, leading to PMN activation, which is further enhanced by the abundance of other pathogens. This activation could lead to excessive and inappropriate reactive oxygen species production by PMN, as well as IL-8 production, which might in turn amplify the phenomenon, leading to oxidative injury of surrounding tissues.

PMN pretreatment in vitro with a substimulatory concentration of a “primer” can enhance the response to a secondary stimulus. This priming phenomenon occurs in response to bacterial N-formyl peptides after pretreatment with a number of cytokines (including TNF-α and IL-8) and endotoxin, and may also occur during endothelial transmigration in vivo. In this way, resting PMN would be preactivated and would thus be able to produce a more powerful bactericidal response at the inflammatory site (44, 46). We and others have reported that fMLP, LPS, TNF-α, and IL-8 alone induce barely detectable H2O2 production by whole-blood PMN from healthy control subjects, whereas pretreatment of blood with TNF-α, LPS or IL-8 strongly primes PMN to produce H2O2 in response to fMLP (26, 47). We confirmed that H2O2 production after TNF-α and LPS priming of PMN from the patients with AP was normal (18). We extended this observation to the other two groups of patients (data not shown). In contrast, H2O2 production after IL-8 priming was strongly decreased in RPP patients. This reduced response to fMLP stimulation after IL-8 priming could be related to PMN desensitization by increased IL-8 plasma levels (48) and could lead to decreased bactericidal activity and proliferation of pathogens in the periodontal inflammatory sites of RPP patients. Professional treatment of gingival inflammation is accompanied by a normalization of IL-8 values, the PMN oxidative burst, and PMN priming by IL-8 in RPP patients (16). In the same way, adhesion molecule expression was normalized by professional reduction of gingival inflammation in the three groups (data not shown). These results suggest a link between these different features of the disease, in which PMN IL-8 production is probably self-amplifying.

In conclusion, PMN from RPP patients showed dysregulated L-selectin shedding and increased basal H2O2 production. These abnormalities, associated with increased IL-8 plasma levels, could be related to P. gingivalis, which was only found in the periodontal pockets of the RPP patients. The decreased IL-8-induced priming of H2O2 production could contribute to defective bactericidal activity, leading to a larger number of pathogens (in addition to P. gingivalis) in RPP than in LJP and AP. These abundant pathogens could also amplify alterations of PMN functions and contribute to vascular and tissue damage. Moreover, decreased sL-selectin plasma levels in RPP patients probably reflect diffuse activation of the endothelium. These abnormalities were not found in the two other categories of patients with less destructive forms of periodontitis (AP and LJP), pointing to a correlation between PMN abnormalities and the clinical form of periodontitis.

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References


