Salivary defense mechanisms in juvenile periodontitis

Leena Saxén, Jorma Tenovuo and Pekka Vilja
Department of Periodontology, University of Helsinki, Helsinki; Department of Cariology, Institute of Dentistry, University of Turku, Turku; and Department of Biomedical Sciences, University of Tampere, Tampere; Finland

Saxén L, Tenovuo J, Vilja P. Salivary defense mechanisms in juvenile periodontitis. Acta Odontol Scand 1990;48:399–407. Oslo. ISSN 0001–6357.

The local, saliva-associated defense mechanisms of 28 juvenile periodontitis (JP) patients and their age- and sex-matched controls were studied. Lysozyme, lactoferrin, salivary peroxidase, myeloperoxidase, and thiocyanate concentrations were determined from both whole saliva and parotid saliva. The total concentrations of salivary IgA, IgG, and IgM were assayed. The periodontal condition and the salivary flow rates were registered. Among the JP patients, a significantly elevated concentration of IgG was found in parotid saliva but not in whole saliva. Salivary peroxidase activities were significantly low both in the whole and in the parotid saliva samples of the JP patients, and leukocyte-derived myeloperoxidase was present in significantly low amounts in whole saliva of these patients. Because both glandular (salivary peroxidase) and polymorphonuclear-cell-derived (myeloperoxidase) enzyme activities were low among the JP patients, suppressed peroxidase-mediated host defense mechanisms could be characteristic of JP. □ *Enzyme activity; periodontology; salivary IgA; salivary IgG; salivary IgM*

Leena Saxén, Department of Periodontology, University of Helsinki, Mannerheimintie 172, SF-00300 Helsinki, Finland

Juvenile periodontitis (JP) is a rapidly progressing inflammatory periodontal disease of otherwise healthy adolescents and young adults. Although the disease was described for the first time more than 60 years ago (1), and explicit clinical criteria were published almost 20 years ago (2), the etiology of JP remains open despite intensive research. A specific subgingival microflora has been suggested to be the main etiologic factor; in particular, *Actinobacillus actinomycetemcomitans* has been associated with JP. *A. actinomycetemcomitans* occurs in at least 90% of cases of subgingival plaque in JP patients, whereas in adult periodontitis the percentage is 20, and in periodontally healthy individuals, 17 (3, 4). *A. actinomycetemcomitans* is a pathogenic, capnophilic, facultatively anaerobic Gram-negative coccobacillus. Among its many virulence factors, *A. actinomycetemcomitans* produces inhibitors to neutrophil chemotaxis (5) and leukotoxins to polymorphonuclear cells (PMNs) (6). However, some reports have not confirmed the association of *A. actinomycetemcomitans* and JP (7, 8). There are, however, indications that it belongs to the normal periodontal flora although present only in low numbers (9), which means that it does not cause disease in all individuals.

Some minor immunologic aberrations have also been suggested to be etiologic factors of JP (10); that is, an impaired chemotaxis of the PMN cells of the peripheral blood has been postulated to occur in 70% of JP patients (11, 12). However, there are studies that do not corroborate these findings in JP patients (13–15). Furthermore, if the chemotactic reactivity is decreased, the defect has to be small, because JP patients should, by definition, be in good general health (2). In addition to the immunologic defense system, many nonspecific antimicrobial factors also operate in the human mouth. These include lysozyme, lactoferrin, peroxidase systems, agglutinins, and histidine-rich proteins (16, 17). The agents are either synthesized in the salivary glands or leak into the mouth from blood, usually via gingival crevices. Many agents (for example, lysozyme, lactoferrin, per-

oxidases) exist both in pure glandular secretions and in crevicular fluid (17). To our knowledge, no previous studies exist of any possible JP-associated changes in these innate antimicrobial factors.

Saliva and gingival crevicular fluid (GCF) are the main environmental components at the dentogingival junction, and the cells of the GCF and their metabolites form an important part of the saliva.

Salivary peroxidase activity has been found to be significantly reduced in mice with Chediak–Higashi syndrome (CH) (18). CH is a genetic disorder also occurring in man. The major oral manifestation is severe periodontal breakdown at a young age, resulting in loss of the teeth (19, 20). There is also a low activity of natural killer cells in both CH and JP patients (21).

Although JP is a local oral disease, the saliva of these patients has virtually not been investigated. We therefore wanted to ascertain whether there were any aberrations in the salivary defense mechanisms in patients with JP.

Materials and methods

The material consisted of 28 patients with JP fulfilling the diagnostic criteria of Baer (2) and their age- and sex-matched, generally and periodontally healthy controls. The age of the subjects ranged between 15 and 38 years (mean, 23.8 years) at the time of the test but between 14 and 29 (mean, 19.3) years at the time of the diagnosis. The M/F sex ratio was 7:21. Three of the JP patients had the generalized type of the disease, and the others the typical first-molar-incisor type of disease (LJP); 23 patients were in the maintenance phase of the treatment, and 5 in the initial treatment phase.

The controls were recruited among the dental students and the nurses of the Dental School and their children.

Gingival condition

The gingival condition of all subjects was assessed by scoring the gingival bleeding on gentle probing to the bottom of the pocket at four sites of each tooth (mesial, distal, buccal, lingual). It was recorded as the percentage bleeding sites of the total number of scored sites per patient.

Probing depth

Probing depths of 4 mm or more were registered for the JP patients only, while none of the controls had a probing depth of that extent. The probing depths were measured at the same sites as the bleeding, and depths of $\geq$4 mm were registered and recorded as a percentage of the total number of sites probed.

Salivary samples

Two milliliters of parotid saliva was collected from each subject with modified Curby cups. To stimulate the salivary flow, a citric acid crystal was placed on the tongue.

The paraffin-stimulated whole saliva was obtained by having the subject spit in a graduated receptacle until the yield was 3 ml. The flow rate (ml/min) was calculated by dividing the yield by the time needed to collect it. Before salivary sampling, the subjects did not eat or drink for 1 h. The samples were taken randomly from JP patients or controls during the study period.

Centrifuged saliva samples were stored at −20°C until analyzed. All assays were done with centrifuged saliva.

Chemical assays

The lysozyme level was estimated with Micrococcus diffusion plates (Lysozyme Kit, Kallestad Laboratories, Chaska, Minn., USA) with lyophilized human urine lysozyme as a standard. Before lysozyme assay the saliva sample was made 0.5 M with NaCl and again centrifuged to release bound lysozyme (22). The lactoferrin level was determined by a noncompetitive avidin-biotin enzyme immunoassay (23). Human colostral lactoferrin (Sigma Chemical Co., St. Louis, Mo., USA), further purified by affinity chromatography, was used as standard.

Salivary peroxidase activity was measured at 22°C by following the rate of oxidation

Table 1. The clinical periodontal status of 28 patients with juvenile periodontitis (JP) and their age- and sex-matched controls (C)

Age, years		GB, %		Probing depth % ≥ 4 mm in JP	State of treatment* of JP
At diagnosis	At test	JP	C		
14	15	18.8	13.4	0	I
15	16	4.5	10.7	0	M
15	16	6.3	12.5	3.6	M
15	17	20.5	8.9	17.9	I
17	18	14.3	4.5	17.2	I
16	19	17.9	13.4	0.9	M
17	19	7.2	0	3.6	M
17	20	0	8.0	2.7	M
19	21	3.6	3.6	0	M
14	21	0	6.3	0	M
16	21	33.0	1.8	1.8	M
22	23	1.8	0.9	1.8	M
23	23	26.8	0.9	2.7	M
17	24	4.2	0	9.4	M
16	25	0.9	0.9	0	M
24	25	24.1	4.5	8.9	I
16	26	8.0	0	0	M
16	26	4.5	6.3	4.5	M
21	26	0	1.8	0	M
16	26	33.9	0	0.9	M
25	26	5.4	4.5	2.7	M
25	26	2.9	0.9	4.8	M
23	26	6.3	0	8.0	M
25	27	28.5	0	20.5	I
17	28	8.9	0.9	4.5	M
27	32	4.6	0	9.3	M
29	35	16.7	3.8	6.0	M
24	38	0	4.5	0	M
$\bar{X}$ 19.3	$\bar{X}$ 23.8	10.8 ± 10.6	4.0 ± 4.4	4.7 ± 5.7	5/23

* I = initial treatment; M = maintenance care.

of 5-thio-2-nitrobenzoic acid (Nbs) to 5,5-dithiobis-(2-nitrobenzoic acid) (Nbs_2) (Aldrich Chemical Co., Milwaukee, Wisc., USA) by hypothiocyanite ($OSCN^-$) ions generated during the oxidation of thiocyanate (SCN^-) by salivary peroxidase (24). Details of the assay system have been described previously (25). The replacement of SCN^- by Cl^- in the assay mixture makes the method suitable for determination of specific myeloperoxidase activity in saliva, since Cl^- is oxidized to OCl^- by myeloperoxidase but not by salivary peroxidase (26).

The $OSCN^-$ levels of saliva were not assayed, because all chemical analyses were made from frozen samples that do not contain detectable $OSCN^-$ (25).

The SCN^- concentrations were determined by the ferric nitrate method (27). The total protein content was measured in accordance with Lowry et al. (28). Amylase activity was determined by the Phadebas method (Pharmacia, Uppsala, Sweden).

Table 2. Flow rate and thiocyanate and total protein concentrations in whole saliva of patients with juvenile periodontitis (JP) and of their age- and sex-matched controls. There were no statistically significant differences between the groups (n = 28 in both groups)

	JP patients		Controls	
	Mean	SD	Mean	SD
Flow rate (ml/min)	1.71	0.85	1.88	0.81
Thiocyanate (mM)	0.98	0.55	0.92	0.42
Protein (mg/ml)	1.29	0.47	1.35	0.37

Table 3. Lysozyme, salivary peroxidase, and amylase activities and lactoferrin and total protein concentrations in parotid saliva of patients with juvenile periodontitis (JP) and of their age- and sex-matched controls (n = 28 in both groups)

	JP patients		Controls		
	Mean	SD	Mean	SD	Significance*
Lysozyme (μg/ml)	13.7	5.5	11.9	2.2	NS
Lactoferrin (μg/ml)	7.4	5.3	5.9	4.0	NS
Salivary peroxidase (mU)	1.1	0.5	1.4	0.6	$p < 0.05$
Amylase (U/l × 10)	407	309	486	263	NS
Protein (mg/ml)	1.8	0.8	1.8	0.6	NS

* Mann–Whitney U-test; NS = not significant.

The total concentrations of salivary IgA, IgG, and IgM, were assayed by means of a 'trapping antibody'-type enzyme immunoassay, as described in detail by Lehtonen et al. (29). Rabbit anti-human IgA, IgG, and IgM and horseradish-peroxidase-conjugated anti-human immunoglobulins (Dako Immunoglobulins A/S, Copenhagen, Denmark) were used in the analysis. The immunoglobulin standards were purified from human serum (Behringwerke AG, Marburg, FRG). The absorbance in the immunoglobulin and lactoferrin analyses was read with an automatic photometer (Titertek Multiscan, Eflab Oy, Helsinki, Finland). The statistical differences between the study group and the control group were analyzed by the Mann–Whitney U-test.

Results

Periodontal health

The clinical status of the JP patients and the controls is presented in Table 1.

Salivary factors

The flow rate of the paraffin-stimulated whole saliva was 1.7 ± 0.9 ml/min (range, 0.7–4.0 ml/min) among the JP patients and 1.9 ± 0.8 ml/min (range, 0.6–4.0 ml/min) among the controls (Table 2). The difference is not significant.

There were no differences in salivary concentrations of lysozyme or lactoferrin between the JP patients and their healthy controls (Fig. 1, Table 3). Especially in whole saliva from the JP patients, the inter-individual variation in the levels of these agents was high (Fig. 1). Salivary peroxidase activities both in parotid and in whole saliva were significantly lower among the JP patients than among the controls (Fig. 2, Table 3); the same was true for whole saliva myeloperoxidase activity (Fig. 2.). No differences between the two groups were observed in salivary total protein content (Tables 2 and 3), whole saliva thiocyanate levels (Table 2), or amylase activities in parotid saliva samples (Table 3).

In general, the salivary concentrations of immunoglobulins (isotypes A, G, and M) were somewhat higher among the JP patients than among the controls, but the difference was statistically significant only for IgG in parotid saliva (Table 4). Age, sex, or the phase of treatment did not affect the results in any systematic manner.

Discussion

As several authors have reported on impaired host defense among patients with JP (10), we wanted to ascertain whether the saliva-mediated antimicrobial systems are also impaired in this disease. There were no differences in flow rate or total protein content between the JP patients and their controls, suggesting that no major changes in the protective functions of saliva were to be expected. Also, the similarity of the

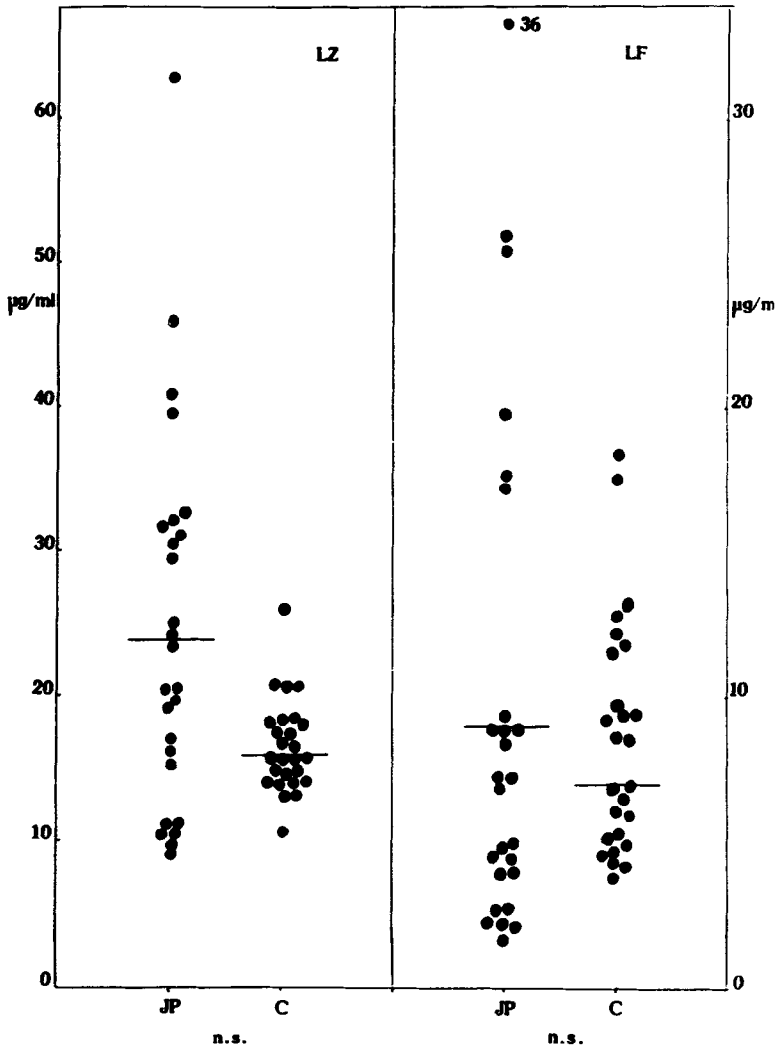

Fig. 1. Lysozyme (LZ) and lactoferrin (LF) concentrations in whole saliva of patients with juvenile periodontitis (JP) and of their age- and sex-matched controls (C). The horizontal lines indicate the mean values; $n = 28$ in both groups.

amylase concentrations in the parotid saliva samples of these two groups indicated no major abnormality in salivary gland protein synthesis.

Many of the antimicrobial agents in whole saliva originate both from pure salivary secretions and from gingival crevicular fluid (17). To facilitate the interpretation of the results, both whole and parotid saliva were collected for analysis. As the control subjects were in good periodontal health, it is most likely that, compared with the JP patients, the contribution of crevicular fluid components to whole saliva among them is small. On the other hand, any differences in parotid saliva composition may reflect a more generalized change in the host defense system of the JP patients. The elevated total IgG response in parotid saliva of the JP patients agrees with the previous results (30).

Lysozyme and lactoferrin are normal antimicrobial constituents of both parotid saliva and gingival fluid (17). Phagocytic cells are rich in these proteins (31), and lysed cells from the exudate of inflamed gingiva may contribute to the number present in whole saliva (32). In our study there were no differences in the lysozyme and lactoferrin concentrations between the study group and the controls, indicating no abnormality in these

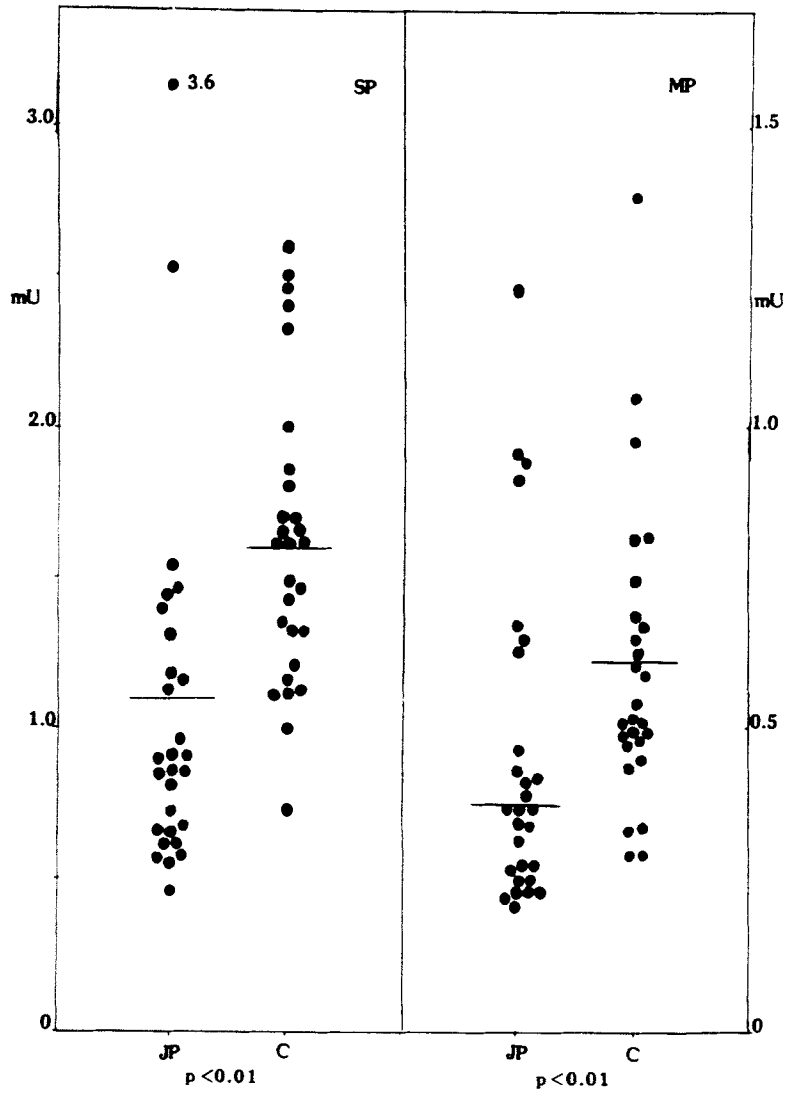

Fig. 2. Whole saliva activities of salivary peroxidase (SP) and myeloperoxidase (MP) in patients with juvenile periodontitis (JP) and in their age- and sex-matched controls (C). The horizontal lines indicate the mean values; n = 28 in both groups.

proteins among the JP patients. However, the salivary levels of both constituents were slightly higher and much more variable among the JP patients, obviously due to the higher degree of gingival inflammation in JP.

The suggested periodontopathogen of JP, *A. actinomycetemcomitans*, is sensitive to both lysozyme- (33) and lactoferrin-mediated (34) killing in vitro. Accordingly, it has been suggested (33, 34) that dysfunction of PMN cells from JP patients in killing *A. actinomycetemcomitans* effectively could be related to defects in phagocytic lysozyme and/or lactoferrin functions or to the reduced number of these cells in the subgingival area due to inhibited neutrophil chemotaxis (12). However, our data show that, at least in saliva, these antimicrobial proteins exist in concentrations comparable with those in healthy individuals. Therefore, in the future it is necessary to study the concentrations of these proteins also in the gingival crevicular fluid of JP patients to clarify their role in host defense.

Interestingly, the peroxidase activities (salivary peroxidase and myeloperoxidase)

Table 4. Concentrations (mg/l) of total IgA, IgG, and IgM in paraffin-stimulated whole saliva and parotid saliva of patients with juvenile periodontitis (JP) and of their age- and sex-matched controls (n = 28 in both groups)

	JP patients		Controls		
	Mean	SD	Mean	SD	Significance*
Whole saliva					
IgA	49.7	31.1	39.7	19.9	NS
IgG	16.4	17.9	12,2	11.5	NS
IgM	4.7	6.0	3.4	2.1	NS
Parotid saliva					
IgA	56.2	31.7	49.2	22.0	NS
IgG	0.92	1.23	0.25	0.14	$p < 0.001$
IgM	4.9	6.6	4.5	4.8	NS

* Mann–Whitney U-test; NS = not significant.

were low in JP patients when compared with the controls. Myeloperoxidase exists only in PMN leukocytes, being one of the major antimicrobial agents of these cells (31). Therefore, the sole source for myeloperoxidase activity in human whole saliva is inflammatory cells, and the activity in gingival exudate and the whole saliva is known to reflect the number of leukocytes entering the oral cavity (35, 36). Oral bacteria effectively release myeloperoxidase from oral leukocytes (37), and whole saliva myeloperoxidase activity seems to be a good measure of oral inflammations, such as periodontal disease (38), phenytoin-associated gingival overgrowth (39), and inflamed fissured tongue (40). *A. actinomycetemcomitans* is sensitive to the oxygen-dependent bactericidal action of myeloperoxidase (41, 42).

The reason for the low myeloperoxidase activity in whole saliva from JP patients is not known. If JP results in reduced numbers of PMN cells (due, for example, to a chemotactic defect) in the gingival area, low myeloperoxidase activity in whole saliva may reflect the low number of these cells. However, as salivary peroxidase activity, derived from the parotid glands, was also significantly reduced among JP patients, these findings together suggest a more general defect in the peroxidative activity of these patients. Interestingly, mice with the Chediak–Higashi syndrome, associated with severe periodontal breakdown, also show significantly reduced salivary peroxidase activity (18).

Why specifically peroxidase enzymes were affected by JP is not known. It should be emphasized that the concentration of thiocyanate (SCN^-) affects the assays of both salivary and myeloperoxidase (26), but there were no differences in the salivary SCN^- concentrations between our study group and the control group. Thus no methodologic explanation can be offered for the differences in the peroxidative activity found in our study.

In human whole saliva, peroxidase enzyme is not a limiting factor for the generation of the antimicrobial oxidation products (43). Thus, low enzyme activity in saliva does not necessarily indicate an impaired antimicrobial capacity of these enzyme systems. This can be further studied by analyzing saliva for $HOSCN/OSCN^-$ in JP; this was not possible in the present study because—for practical reasons—saliva samples had to be frozen before the assays. However, low peroxidase activity in saliva or gingival crevicular fluid may be disadvantageous to periodontal tissues because these enzymes have an important role in protecting mucosal cells of the oral cavity from H_2O_2 toxicity (44). Hydrogen peroxide at very low concentrations (10–100 μM) kills human gingival fibroblasts (45, 46), and H_2O_2 concentrations below this critical level are maintained in the mouth by peroxidase enzymes (43). Hydrogen peroxide, which is

constantly produced by many oral bacteria and by leukocytes (44), is converted by peroxidases to form dioxygen, O_2 (catalatic activity), or HOSCN/$OSCN^-$ via oxidation of SCN^- ions (47). These products are not toxic to gingival cells (44).

Acknowledgements.—Financial support from the Finnish Dental Society and the Helsinki University Research Fund and the Academy of Finland are gratefully acknowledged. The authors thank Mrs. Pirjo Mäkijouppila for collecting the saliva samples.

References

1. Gottlieb B. Die diffuse Atrophie des Alveolarknochens weitere Beiträge zur Kenntnis des Alveolarschwundes und dessen Wiedergutmachung durch Zementwackstum. Z Stomatol 1923;21:195–262.
2. Baer PN The case for periodontosis as a clinical entity. J Periodontol 1971;42:516–9.
3. Zambon JJ, Christersson LA, Slots J. *Actinobacillus actinomycetemcomitans* in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. J Periodontol 1983;54:707–11.
4. Asikainen S, Jousimies-Somer H, Kanervo A, Saxén L. *Actinobacillus actinomycetemcomitans* and clinical periodontal status in Finnish juvenile periodontitis patients. J Periodontol 1985;57:91–3.
5. Van Dyke TE, Bartholomew E, Genco RJ, Slots J, Levine MJ. Inhibition of neutrophil chemotaxis by soluble bacterial products. J Periodontol 1982; 53:502–8.
6. Baehni PC, Tsai CC, McArthur WP, Hammon BF, Taichman NS. Interaction of inflammatory cells and oral micro-organisms. VIII. Detection of leukotoxic activity of a plaque-derived gram-negative microorganism. Infect Immun 1979;24:233–43.
7. Vandesteen GE, Williams BL, Ebersole JL, Altman LDC, Page RC. Clinical, microbiological and immunological studies of a family with a high prevalence of early-onset periodontitis. J Periodontol 1984;55:159–69.
8. Moore WEC, Holdeman LV, Cato EP, et al. Comparative bacteriology of juvenile periodontitis. Infect Immun 1985;48:507–19.
9. Kilian M, Schiøtt CR. Haemophili and related bacteria in the human oral cavity. Arch Oral Biol 1975;20:791–6.
10. Sandholm L. The cellular host response in juvenile periodontitis. A review. J Periodontol 1985;56:359–66.
11. Cianciola LJ, Genco RJ, Patters MR, McKenna J, van Oss CJ. Defective polymorphonuclear leukocyte function in a human periodontal disease. Nature 1977;265:445–7.
12. Van Dyke TE, Horoszewicz HV, Genco RJ. The polymorphonuclear leukocyte (PMNL) locomotor defect in juvenile periodontitis—study of random migration, chemokinesis and chemotaxis. J Periodontol 1982;53:682–7.
13. Larjava H, Saxén L, Kosunen T, Gahmberg CG. Chemotaxis and surface glycoproteins of neutrophil granulocytes from patients with juvenile periodontitis. Arch Oral Biol 1984;11:935–9.
14. Kinane DF, Cullen CF, Johnston FA, Evans CW. Neutrophil chemotactic behaviour in patients with early-onset forms of periodontitis. I. Leading front analysis in Boyden chambers. J Clin Periodontol 1989;16:242–6.
15. Kinane DF, Cullen CF, Johnston FA, Evans CW. Neutrophil chemotactic behaviour in patients with early-onset forms of periodontitis. II. Assessment using the under agarose technique. J Clin Periodontol 1989;16:247–51.
16. Mandel ID, Ellison SA. The biological significance of the nonimmunoglobulin defense factors. In: Pruitt KM, Tenovuo J, eds. The lactoperoxidase system: chemistry and biological significance. New York: Marcel Dekker Inc., 1985:1–14.
17. Tenovuo J, Gråhn E, Lehtonen O-P, Hyyppä T, Karhuvaara L, Vilja P. Antimicrobial factors in saliva: ontogeny and relation to oral health. J Dent Res 1987;66:475–9.
18. Hiramatsu M, Kashimata M. Peroxidase activity in salivary glands and saliva of beige (Chediak-Higashi) mice. Arch Oral Biol 1985;30:753–5.
19. Hamilton RE, Giansanti JS. The Chediak-Higashi syndrome: report of a case and review of the literature. Oral Surg 1974;37:754–61.
20. Lavine WS, Page RC, Padgett GA. Host response in chronic periodontal disease. V. The dental and periodontal status of mink and mice affected by Chediak-Higashi syndrome. J Periodontol 1976; 47:621–35.
21. Saxén L, Saksela E. Natural killer cell activity in patients with juvenile periodontitis. In: Lehner T, Cimasoni G, eds. Borderland between caries and periodontal disease. III. Geneva: Editions Médicine et Hygiène, 1986:291–6.
22. Jenzano J, Hogan SL, Lundblad RL. Factors influencing measurement of human salivary lysozyme in lysoplate and turbidimetric assays. J Clin Microbiol 1986;24:963–7.
23. Vilja P, Krohn K, Tuohimaa P. A rapid and sensitive non-competitive avidin-biotin assay for lactoferrin. J Immunol Meth 1985;76:73–83.
24. Wever R, Kast WM, Kasinoedin JH, Boylens R. The peroxidation of thiocyanate catalysed by myeloperoxidase and lactoperoxidase. Biochim Biophys Acta 1982;709:212–9.
25. Tenovuo J, Lehtonen O-P, Aaltonen AS, Vilja P, Tuohimaa P. Antimicrobial factors in whole saliva of human infants. Infect Immun 1986;51:49–53.
26. Mansson-Rahemtulla B, Baldone DC, Pruitt KM, Rahemtulla F. Specific assays for peroxidases in human saliva. Arch Oral Biol 1986;31:661–8.
27. Betts RH, Dainton FS. Electron transfer and other processes involved in the spontaneous bleaching of acidified solutions of ferric thiocyanate. J Am Chem Soc 1953;75:5721–7.

28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–72.
29. Lehtonen O-P, Gråhn E, Ståhlberg T, Laitinen LA. Amount and avidity of salivary and serum antibodies against Streptococcus mutans in two groups of human subjects with different dental caries susceptibility. Infect Immun 1984;43:308–13.
30. Sandholm L, Grönblad E. Salivary immunoglobulins in patients with juvenile periodontitis and their healthy siblings. J Periodontol 1984;55:9–12.
31. Spitznagel JK. Antibacterial mechanisms of polymorphonuclear leukocytes. In: Kleinberg I, Ellison SA, Mandel ID, eds. Saliva and dental caries. Microbiol Abstracts 1979(spec suppl); 391–411.
32. Gråhn E, Tenovuo J, Lehtonen O-P, Eerola E, Vilja P. Antimicrobial systems of human whole saliva in relation to dental caries, cariogenic bacteria, and gingival inflammation in young adults. Acta Odontol Scand 1988;46:67–74.
33. Iacono VJ, Boldt PR, MacKay BJ, Cho M-I, Pollock JJ. Lytic sensitivity of *Actinobacillus actinomycetemcomitans* Y4 to lysozyme. Infect Immun 1983;40:773–84.
34. Kalmar JR, Arnold RR. Killing of *Actinobacillus actinomycetemcomitans* by human lactoferrin. Infect Immun 1988;56:252–7.
35. Basu MK, Smith AJ, Walsh TF, Caddick CE. Changes in salivary peroxidase activity during experimentally induced gingivitis [Abstract]. J Dent Res 1982;61:560.
36. Klinkhamer JM, Mitchell MD. Orogranulocyte peroxidase activity as a measure of inflammatory periodontal disease. J Dent Res 1979;58:531–4.
37. Taichman NS, Tsai C-C, Baehni P, Stoller N, McArthur WP. Interaction of inflammatory cells and oral microorganisms. IV. In vitro release of lysosomal constituents from polymorphonuclear leukocytes exposed to supragingival and subgingival bacterial plaque. Infect Immun 1977;16:1013–23.
38. Smith QT, Hinrichs JE, Melnyk RS. Gingival crevicular fluid myeloperoxidase at periodontitis sites. J Periodont Res 1986;21:45–55.
39. Smith QT, Hamilton MJ. Salivary composition, phenytoin ingestion and gingival overgrowth. J Periodontol 1981;52:673–9.
40. Kullaa-Mikkonen A, Tenovuo J, Sorvari T. Changes in composition of the whole saliva in patients with fissured tongue. Scand J Dent Res 1985;93:522–8.
41. Miyasaki KT, Wilson ME, Brunetti AJ, Genco RJ. Oxidative and nonoxidative killing of *Actinobacillus actinomycetemcomitans* by human neutrophils. Infect Immun 1986;53:154–60.
42. Miyasaki KT, Wilson ME, Genco RJ. Killing of *Actinobacillus actinomycetemcomitans* by the human neutrophil myeloperoxidase-hydrogen peroxide-chloride system. Infect Immun 1986;53:161–5.
43. Pruitt KM, Tenovuo J, Fleming W, Adamson M. Limiting factors for the generation of hypothiocyanite ion, an antimicrobial agent, in human saliva. Caries Res 1983;16:315–23.
44. Carlsson J. Salivary peroxidase: an important part of our defense against oxygen toxicity. J Oral Pathol 1987;16:412–6.
45. Hänström L, Johansson A, Carlsson J. Lactoperoxidase and thiocyanate protect cultured mammalian cells against hydrogen peroxide toxicity. Med Biol 1983;61:268–74.
46. Tenovuo J, Larjava H. The protective effect of peroxidase and thiocyanate against hydrogen peroxide toxicity assessed by the uptake of H-thymidine by human gingival fibroblasts cultured in vitro. Arch Oral Biol 1984;29:445–51.
47. Pruitt KM, Tenovuo J, Mansson-Rahemtulla B, Harrington P, Baldone DC. Is thiocyanate peroxidation at equilibrium in vivo? Biochim Biophys Acta 1986;870:385–91.

Received for publication 16 November 1989