Bacterial Profiles of Subgingival Plaques in Periodontitis*

W. J. Loesche,† S. A. Syed,‡ E. Schmidt§ and E. C. Morrison‖

IN THIS REPORT OVER 400 subgingival plaque samples taken from over 110 patients were examined microscopically and culturally for 30 bacterial parameters. The patients could be placed into six disease categories based upon clinical criteria. The bacterial profile of each clinical category was generally distinctive of that category. Periodontal patients who had been successfully treated and maintained had plaques that were populated by significantly higher proportions of *Streptococcus sanguis, Actinomyces viscosus, A. odontolyticus* and *S. mutans* and significantly lower proportions of *B. gingivalis* and spirochetes compared to the five untreated disease categories. The spirochetes were the overwhelming microbial type in the plaques of adult periodontitis (AP) patients, averaging about 45% of the microscopic count. The bacteriological results could not distinguish between ADA Type III and IV periodontitis, suggesting that the same type of infection was occurring in an active site in any AP patient. The patients designated as early onset periodontitis (EOP) differed from the other patients by their relative youth and by their significantly higher proportions of *Bacteroides gingivalis* and/or *B. intermedius*. Two types of EOP were recognized in which the most diseased variant was characterized by having an average of 49% spirochetes in the plaque. Four localized juvenile periodontitis (LJP) patients were notable in not having detectable *A. actinomycetemcomitans*. The data indicate that the various types of periodontitis, with the possible exception of LJP are specific anaerobic infections involving spirochetes and to a lesser extent *B. gingivalis* and *B. intermedius*.

Periodontal disease is the foremost cause of tooth loss in adult populations.[1] In recent years considerable, but not unequivocal, data have appeared which suggest that some, if not all, forms of periodontal disease may be related either to the overgrowth or to the presence of one or more bacterial types in subgingival plaques removed from discrete tooth sites.[2-5] The majority of this evidence is based upon cross-sectional association studies in which either the bacterial flora on diseased and nondiseased tooth surfaces are compared[6-10] or the serum antibody titers to selected organisms in periodontally healthy or diseased individuals[12-14] are compared.

Two general bacteriological approaches have been used to define the periodontopathic plaque flora. One is to subculture all or representative isolates from a given plaque sample and to perform as many taxonomic tests as are necessary in order to give the isolate a genus or species designation. This approach is necessary to define the plaque flora and has demonstrated an unusual amount of bacterial diversity within the plaque ecosystem[8,10] with the concomitant recognition of many new species.[15-17] However, only a few plaque samples can be so thoroughly characterized. Due to this, general statements concerning the role of any particular species in periodontal disease may be premature.

The other approach is to survey a large number of plaques for the presence of certain easily identifiable organisms, such as the black-pigmented Bacteroides (BPB), spirochetes, *Capnocytophaga* species, *Fusobacterium nucleatum, Actinomyces viscosus* and *Actinobacillus actinomycetemcomitans*, among others. This approach is informative if the identified organisms are either prominent members of the plaque flora, and/or if they contribute meaningfully to the health or disease potential of the plaque, as it permits microbial patterns to be recognized. If the sample size is large enough, statistical tests can be performed relative to both the tooth site and to the patient, thereby overcoming the objections raised when only sites nestled in the same patient are used in the analysis.[18] Conversely, this approach would not identify a heretofore unknown periodontopathic organism unless by chance it was a con-

spicuous organism on the various isolation media and was also enumerated.

The purpose of the present study was to examine a large number of subgingival plaques removed from untreated and successfully treated periodontal patients, in order to determine whether a characteristic bacterial profile could be identified that could be correlated with the clinical appearance of the periodontium in these patients. The patients were placed into six disease categories based upon clinical criteria and the bacterial profile of each clinical category was generally distinctive of that category.

MATERIALS AND METHODS

Classification of Periodontal Categories. Clinical and bacteriological data were collected from over 110 individuals who showed clinical and/or radiographic evidence of periodontal disease, as well as from 12 treated and maintained patients whose periodontal health had been stabilized for at least 1 year or more. All teeth, including molars with furcation involvement, were scored for pocket depth (probing depth) and loss of clinical attachment by one individual using procedures previously described.[19] All information was used to assign the individuals to one of six clinical categories, using as a reference the American Dental Association (ADA) periodontal classification scheme.[20] These clinical categories in turn served as the entities among which the bacteriological variables were compared.

Bacteriological Procedures. Subgingival plaque was usually removed from one pocket per quadrant in each individual. The site chosen appeared, from a clinical and radiographic examination, to be the most severely involved in each quadrant and was usually about molar teeth, i.e., 259 of 423 sampled sites were molars. The supragingival plaque about the sampled site was removed and discarded. The root surface was then scaled with a curette and the adherent plaque on the scaler tip was transferred to a vial containing 0.5 ml of reduced transport fluid (RTF) without EDTA.[21] The plaque samples were placed immediately into an anaerobic chamber and after dispersing for 20 seconds with a Vortex mixer, a 50-μl portion of each sample was removed for microscopic examination. This degree of dispersal separated most of the plaque aggregates without lysing the spirochetes. The remaining sample was diluted to 4 ml, sonically dispersed for 20 seconds with a Kontes sonifier,* serially diluted in RTF and plated automatically with a spiral plater on a variety of media. In this manner microscopic and cultural counts were obtained on the same plaque sample.

The total anaerobic count, the count of BPB species, *Capnocytophaga* species, *F. nucleatum* and *A odontolyticus* were obtained by identification of their distinctive colonies on enriched trypticase soy agar (ETSA).[22]

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* Kontes Glass Co., Vineland, NJ.

Either all or representative colonies of BPB were identified as *Bacteroides gingivalis, B. intermedius* and *B. melaninogenicus* initially by their ability to use glucose, to hydrolyze esculin and to produce indole, and subsequently by rapid tests using chromogenic enzyme substrates.[23,24] *A. viscosus* and *A. naeslundii* were differentiated by catalase activity of colonies growing on a selective GMC[25] or CFAT agar.[26] The total counts of facultative organisms, *Streptococcus sanguis* and *S. mutans* were obtained from growth on ETSA agar containing 2% sucrose and 20 μg/ml of metronidazole, which was incubated anaerobically. *Veillonella* colonies were enumerated on a medium containing MM10 base, minus blood but supplemented with lactate, vancomycin and a 0.004% bromocresol purple indicator.[27]

Ten μl of the aliquot removed for microscopic examination was placed on a glass slide, covered with a 22 × 30 mm cover slip, sealed and viewed by dark-field microscopy. Either 200 organisms or the number of organisms in 20 high-power fields (hpf) were enumerated, depending on which event occurred first. The single cells were identified as spirochetes, selenomonads, vibrio-like motile rods, fusiforms, nonmotile rods or cocci. The spirochetes were further subdivided into large, intermediate and small-size.[4]

Statistical Analysis. The counts for each organism enumerated culturally and microscopically were recorded on forms suitable for computer processing. The proportions of these organisms were calculated by dividing the viable count of the specific organism by the total viable count on the ETSA agar plate in the case of the cultural data, and by dividing the microscopic count of the specific organism by the total microscopic count in the case of microscopic data. If there were less than 20 total colony-forming units (CFU) on the ETSA plate or in the microscopic count, no proportions were calculated. This was to eliminate distortions of the data which can occur when the denominator is very low. This was the case for microscopic counts in the treated and maintained patients.

The total counts and proportions of organisms in each clinical entity were statistically analyzed by an analysis of variance using the Scheffe test to compare between the clinical groups, and by the nonparametric Kruskal-Wallis test. The data were compared on a per patient basis by averaging the findings of 3 to 5 plaque samples removed from the patient so as to give an average value for each patient (total number = 112) or by treating each plaque sample as a separate entity (total number = 423). In the latter analysis it is assumed that the tooth site and not the patient is the meaningful biological point of reference.

RESULTS

The patients were placed into six distinct categories based upon clinical criteria (Table 1). The actual patient

numbers vary somewhat in the tables as in a few patients either the microscopic or the cultural data were lost due to methodological problems. The 12 treated and maintained patients differed from the untreated patients in having fewer pockets greater than 3 mm. The residual morbidity of their periodontal condition was evident in the high number of sites with attachment loss of 4 to 6 mm (Table 1). The treated and maintained patients included both ADA Type III and IV periodontal conditions. Four teenagers had the distinctive clinical appearance of localized juvenile periodontitis (LJP) with deep pockets and clinical attachment loss primarily about first molars.

A third group of patients was characterized by the clinical impression that an "active" process was ongoing. Similar patients have been reported by other investigators and given names such as "rapidly progressive,"[28] "severe,"[10] "advanced destructive"[14] or "generalized juvenile"[29] periodontitis. These patients will be referred to as early onset periodontitis (EOP) and could be subdivided into two groups called Types A and B. The five Type A patients, despite being somewhat older, exhibited an earlier stage of periodontitis, as there were essentially no pockets or attachment loss greater than 6 mm. They could be considered to be ADA Type II patients. In contrast, the Type B patients exhibited on the average 19 sites with pocket depths greater than 6 mm and 15 sites with attachment loss greater than 6 mm (Table 1). Bleeding upon probing was common in both types of patients.

A fourth group of patients was characterized as being "adult" periodontitis (AP). It was separated into two subgroups compatible with the ADA classification scheme for Type III and Type IV periodontitis.[20] The Type IV patients were the oldest of the untreated patients and had significantly more sites with attachment loss greater than 6 mm. They were the most common patient group encountered, comprising 64% of the patients in our study population. The EOP Type B patients could be considered, on the basis of probing characteristics, as being Type IV patients, but their youth, the clinical impression and the bacteriological profile (see below) indicated that they could be recognized as a separate entity.

The clinical characteristics of the sites sampled for the bacteriological analysis are given in Table 2. The depicted values are averages of 3 to 5 sites per patient and reflected the clinical distinctions between the six groups as described for the whole mouth (Table 1). Thus, the sampled pockets in the treated and maintained patients had probing depths that were significantly lower than the pocket depths in the five untreated categories (Table 2, Scheffe comparison $P = 0.01$). The sampled pockets in the Type A EOP patients differed significantly from the sampled pockets in the Type B EOP patients in regard to pocket depth and attachment loss (Table 2). The deepest pockets were in the Type B EOP patients and in the LJP patients, whereas the sampled pockets with the greatest attachment loss were found in the Type IV patients. There were no significant differences in any of the measured *clinical* parameters between the Type B and Type IV pockets underlining the fact that these probing-derived parameters could not distinguish between the two types of patients.

Cultural studies revealed bacteriological differences between the EOP Types B and IV patients, in that the

Table 1
Clinical Characteristics of Patients Grouped According to Periodontal Morbidity and Age

| | Treated and maintained | Clinical Diagnosis of Periodontitis | | | | |
| | | Early onset | | Adult | | LJP |
		A	B	III	IV	
No. of patients	12	5	18	14	64	4
Age in years	46 ± 10*	30 ± 12†	22 ± 5	34 ± 4†	44 ± 11†	16 ± 4†·**
ADA class	III and IV	II	IV	III	IV	
Total teeth	26 ± 2	25 ± 3	26 ± 4	24 ± 3	24 ± 4	27 ± 0.4
Pocket depth†						
≤3 mm	109 ± 11†	79 ± 14	63 ± 18	73 ± 23	54 ± 22	102 ± 12
4–6 mm	20 ± 12	45 ± 24	47 ± 19	41 ± 13	48 ± 17	24 ± 13
7–9 mm	0.3 ± 0.5	0.4 ± 0.5	19 ± 10	5 ± 4	16 ± 14	8 ± 2
≥10 mm	0	0	1 ± 1	0	1 ± 2	0
Attachment loss†						
≤3 mm	88 ± 19†	107 ± 8	80 ± 25	68 ± 37	42 ± 27	129 ± 20
4–6 mm	38 ± 19	17 ± 23	35 ± 24	44 ± 26	48 ± 17	6 ± 2
7–9 mm	3 ± 4	0.2 ± 0.4	15 ± 14	7 ± 4	⟦26 ± 16⟧	0.3 ± 0.6
≥10 mm	0	0	1 ± 2	0	3 ± 4	0

▭ Value in box is significantly different from all values in row.

† Number of sites per patient. Value with ** is significantly different from all other values in row with same superscript.

* Average ± standard deviation.

Table 2
Clinical Characteristics of Sampled Pocket Sites (Average Values) in Patients Grouped According to Whole-Mouth Clinical Criteria

	Whole-mouth clinical diagnosis of periodontitis					
	Treated and maintained	Early onset		Adult (ADA)		Localized juvenile
		A	B	III	IV	
No. of patients	12	5	18	14	64	4
Pocket depth (mm)	3.9 ± 1*	4.9 ± 1†	7.0 ± 1.6†	5.4 ± 1.2	6.1 ± 1.4	6.8 ± 0.8
Attachment loss (mm)	3.7 ± 0.9	3.0 ± 0.8†	6.1 ± 2.1†	5.6 ± 0.8	6.7 ± 1.4	4.5 ± 1.6

☐ Value in row significantly different from all other values in row, Scheffe Test *P* < 0.05.
* All values are an average of 3 to 5 pocket sites per patient.
† Values in same row with same superscript are significantly different.

Table 3
Bacterial Characteristics of Plaque from Sampled Pocket Sites (Average Value per Patient)

	Clinical diagnosis of periodontitis						Significance— ANOVA*
	Treated and maintained	Early onset		Adult (ADA)		Localized juvenile	
		A	B	III	IV		
No. of patients	11	5	18	16	67	4	121
Total count × 10⁶ CFU	6 ± 5†·**	53 ± 71	38 ± 43†	19 ± 20†	23 ± 35†	21 ± 30	NS‡
% B. intermedius	4 ± 6	10 ± 10	11 ± 11	6 ± 4	8 ± 8	5 ± 4	NS
% B. gingivalis	0.2 ± 0.2	14 ± 15	13 ± 17	2 ± 7†	4 ± 9†	<0.01†·**	p = 0.003
% B. melaninogenicus	0.1 ± 0.2†	≤0.01	2 ± 3†·**	0.7 ± 7†	0.7 ± 1†	0.1 ± 0.1	0.06
% F. nucleatum	3 ± 3	3 ± 2	3 ± 2	4 ± 3	4 ± 4	4 ± 4	NS
% Selenomonas sp.	ND§	ND	1 ± 2	2 ± 2	2 ± 3	1 ± 2	NS
% Veillonella	4 ± 3	2 ± 2	2 ± 3	0.7 ± 0.2	0.8 ± 1	4 ± 6	0.001
% Actinobacillus sp.	ND	ND	<0.01	<0.01	<0.01	<0.01	NS
% A. odontolyticus	2 ± 3†·**	1 ± 1	0.4 ± 0.8†	0.3 ± 0.6†	1 ± 2†	0.3 ± 0.4†	NS
% A. viscosus	6 ± 4	0.5 ± 0.4	1 ± 2	2 ± 4	3 ± 3	2 ± 2	0.005
% A. naeslundii	0.5 ± 0.6	0.3 ± 0.5	1 ± 3	1 ± 2	1 ± 2	1 ± 2	NS
% S. sanguis	14 ± 16	0.9 ± 0.3	2 ± 2	0.8 ± 0.8	2 ± 2	3 ± 4	0.001
% S. mutans	3 ± 3†·**	1 ± 1	0.1 ± 0.1†	0.2 ± 0.3†	0.5 ± 1†	3 ± 5†·**	0.001
% Capnocytophaga sp.	2 ± 2	2 ± 3	2 ± 2	2 ± 1	2 ± 2	1 ± 1	NS
% Facultative	39 ± 10†·**	21 ± 24	26 ± 18†	51 ± 43†	35 ± 23	22 ± 15†	0.07

☐ Value is significantly different from all other values in row—Scheffe.
* Analysis of variance.
† Value with ** is significantly different from other values in row with same superscript, i.e., *P* < 0.05, Scheffe contrast.
‡ NS, not significant.
§ ND, not determined.

plaque removed from the Type B pockets had significantly higher proportions of *B. gingivalis, B. melaninogenicus* and *Veillonella* sp. relative to the pockets in the Type IV patients (Table 3). The high proportions of *B. gingivalis* appeared to be pathognomonic in the EOP patients, as both Types A and B plaques had significantly higher proportions of *B. gingivalis* relative to the four other clinical categories (Table 3). Types A and B patients could be distinguished from each other microscopically in that the Type A plaque had significantly lower proportions of total spirochetes and small spirochetes, and significantly higher proportions of cocci (Table 4).

A unique bacterial profile was found in plaques removed from the treated and maintained patients, in that they differed in seven cultural parameters (Table 3) and in two microscopic parameters (Table 4) from the plaques removed from the other clinical categories.

In particular, the proportions of *A. viscosus* and *S. sanguis* were significantly higher than those found in any other clinical category. The proportions of *S. mutans, A. odontolyticus* and total facultative organisms were significantly higher than those values found in at least three of the other categories (Table 3). The proportions of spirochetes (Table 4) and *B. gingivalis* (Table 3) were significantly lower than those values found in the untreated categories. The spirochetes could only be detected in 12 of 28 plaques and *B. gingivalis* in 10 of 44 plaques examined. There was less plaque present in these pockets, as judged by the low total viable count (Table 3) and low numbers of bacteria per hpf (Table 4).

The plaque removed from the LJP patients was distinctive in not having any detectable *B. gingivalis* or *A. actinomycetemcomitans* isolates. This plaque tended to have higher proportions of *S. mutans* and lower pro-

Table 4
Microscopic Characteristics of Plaque from Sampled Pocket Sites (Average Value per Patient)

		Clinical diagnosis of periodontitis					Significance— ANOVA*
	Treated and maintained	Early onset		Adult (ADA)		Localized juvenile	
		A	B	III	IV		
No. of patients	7	4	14	16	65	4	110
Bacteria/hpf†	0.9 ± 0.7	36 ± 29	25 ± 18	16 ± 10	17 ± 10	27 ± 31	$P = 0.001$
% Spirochetes	12 ± 9‡ **	18 ± 12	49 ± 18‡	44 ± 16‡	49 ± 20‡	33 ± 31‡	0.001
% large	ND§	2 ± 3	8 ± 6	4 ± 5	7 ± 7	6 ± 7	NS‖
% intermediate	ND	8 ± 9	13 ± 6	10 ± 7	12 ± 8	15 ± 15	NS
% small	ND	8 ± 3	27 ± 13	30 ± 15	31 ± 13	12 ± 15	0.002
% Selenomonas	4 ± 5	7 ± 3	7 ± 5	11 ± 4	10 ± 10	3 ± 3	NS
% Vibrio-like	5 ± 6	4 ± 1	4 ± 4	3 ± 3	3 ± 4	3 ± 3	NS
% Motile	ND	ND	1 ± 2	6 ± 7	3 ± 3	ND	NS
% Fusiforms	9 ± 5	6 ± 2	5 ± 4	6 ± 4	5 ± 5	14 ± 13	0.01
% Rods	ND	30 ± 5	19 ± 13	21 ± 11	20 ± 10	12 ± 12	NS
% Cocci	ND	36 ± 15	14 ± 15	10 ± 9	11 ± 10	18 ± 15	0.002

☐ Values in box significantly different from all other values in row.
* Analysis of variance.
† hpf, high power field.
‡ Value with ** is significantly different from other values in same row with superscript.
§ ND, not determined.
‖ NS, not significant.

portions of facultative organisms relative to the other disease categories.

The plaques removed from pockets in the AP patients (Types III and IV) were characterized by very high proportions of spirochetes (Table 4) and modest proportions of *B. gingivalis* (Table 3). The proportions of *Veillonella* species were low in the chronic periodontitis patients. None of the monitored bacteriological criteria could distinguish the Type III patient from the Type IV patient, suggesting that the bacterial infection in these pockets was comparable.

The statistical analyses were repeated using the plaque sample as the statistical entity, rather than the patient. Despite the fact that the number of observations increased about fourfold, there were few changes in the values of most parameters and in the number of differences between groups that were significant (data not shown). Among the latter, the cultural data revealed that the EOP Type B plaques had significantly higher proportions of *B. intermedius* than did the plaques found in the Type III, the LJP and the treated and maintained patients. Also, the proportions of *B. intermedius* in the plaques of the treated and maintained patients were significantly less than those found in the EOP and Type IV plaques.

These microbiological findings showed that among the monitored bacterial types or species, elevated proportions of spirochetes, *B. gingivalis* and to a lesser extent *B. intermedius* could be significantly associated with one or more types of periodontitis, excluding LJP. The data upon which this conclusion was based consisted of average values for each bacterial parameter compared across clinical categories with either the patient or the pocket site serving as the statistical entity. Not all patients were infected with these organisms, as

can be observed by examining the frequency distribution of these bacterial types within each clinical category.

Spirochetes were the most common morphological type encountered in the study population, with the only exception being the treated and maintained patients. In these treated patients spirochetes were not detected in seven of 11 patients and when present, ranged from 11 to 27% of the microscopic count. Otherwise, the spirochetes were the dominant microscopic type found in 101 of 103 untreated patients averaging over 40% of the flora in 93% of the EOP Type B patients, in 75% of the Type III patients, and in 85% of the Type IV patients (Table 5).

B. gingivalis was not detected in about half the study population and not at all in the LJP patients (Table 6). None of the treated patients had *B. gingivalis* at levels above 1% of the viable count. Most of the EOP patients, however, had high proportions of *B. gingivalis* (Table 6). When *B. gingivalis* was not detected in these EOP patients, the plaques always had high proportions of *B. intermedius*. In fact, the pattern observed suggested that *B. gingivalis* and *B. intermedius* were mutually incompatible, a possibility that seemed to be verified from *in vitro* testing of these organisms, which showed that some *B. gingivalis* strains could inhibit the growth of *B. intermedius* and a few *B. intermedius* strains could inhibit certain strains of *B. gingivalis* (unpublished results).

B. intermedius was detected in 117 of 123 patients, and in this regard was about as frequently encountered as the spirochetes (Table 7). Unlike the spirochetes, *B. intermedius* was present in all the treated and maintained patients and in four of these patients was present in high proportions (Table 7). It also was a prominent

Table 5

Frequency Distribution of Spirochetes in Patients of each Clinical Category

Disease category	No. of patients	% of Patients in whom spirochetes were				
		Not detected	<10% of micro- scopic count*	10–20% of micro- scopic count	21–40% of micro- scopic count	>40% of micro- scopic count*
Treated and maintained	11	64	0	9	27	0
EOP† Type A	4	0	0	50	0	50
Type B	14	0	0	0	7	93
AP† III	16	0	0	6	19	75
IV	65	0	3	0	12	85
LJP†	4	25	0	25	50	0

* Patients were placed in the percentage range that corresponded with the highest percentage found in any single site in that mouth, i.e., if 4 sites in one patient were 5%, 15%, 22% and 42% spirochetes, the patient was placed in the >40% column.

† EOP, early onset periodontitis; AP, adult periodontitis; LJP, localized juvenile periodontitis.

Table 6

Frequency Distribution of B. gingivalis in Patients for each Disease Category

Disease category	No. of patients	% of patients in whom B. gingivalis was		
		Not detected	≤10% of viable count	>10% of viable count*
Treated and maintained	14	42	58	0
EOP† Type A	5	20	.0	80
Type B	18	39	0	61
AP† III	17	65	29	6
IV	67	51	28	21
LJP†	4	100	0	0

* If one or more sites in a patient had >10% *B. gingivalis* then the patient was counted as having >10% *B. gingivalis*.

† EOP, early onset periodontitis; AP, adult periodontitis; LJP, localized juvenile periodontitis.

Table 7

Frequency Distribution of B. intermedius in Patients of each Clinical Category

Disease category	No. of patients	% of Patients in whom B. intermedius was		
		Not detected	≤10% of viable count	>10% of viable count*
Treated and maintained	12	0	67	33
EOP† Type A	5	20	0	80
Type B	18	22	11	67
AP† III	17	0	53	47
IV	67	3	42	55
LJP†	4	0	50	50

* If one or more sites in a patient had >10% *B. intermedius*, then the patient was counted as having >10% *B. intermedius*.

† EOP, early onset periodontitis; AP, adult periodontitis; LJP, localized juvenile periodontitis.

organism in two of the LJP patients. The EOP group was distinctive in having patients who were either highly infected with *B. intermedius* or were not detectably colonized by this organism (Table 7).

DISCUSSION

The present study of over 400 subgingival plaque samples removed from over 100 untreated periodontal patients and 12 treated and maintained patients indi-cate that sufficient microbial parameters exist which can supplement the clinical parameters to allow the delineation of at least 6 categories of periodontal health and disease.

The treated and maintained group of patients had the most distinctive bacteriological profile in that their plaques were populated by significantly higher propor-tions of *S. sanguis, A. viscosus, A. odontolyticus* and *S. mutans* and significantly lower proportions of *B. gin-*

givalis and spirochetes compared to the five untreated disease categories (Tables 3–7). This combination of elevated proportions of facultative, plaque-forming bacteria and decreased proportions of anaerobic bacteria and spirochetes would seem to constitute the profile of a nondisease-associated plaque, the existence of which was postulated by the specific plaque hypothesis.[2] The low proportions or absence of spirochetes and the high proportions of cocci and rods have been described in previous microscopic studies of plaque removed from relatively healthy sites[4,30] and has been shown to be the flora which established following successful treatment.[31,32]

The present study thus confirmed the previous findings and extended them by adding cultural studies, which identified some of the rods and cocci as *A. viscosus*, *A. odontolyticus*, *S. sanguis* and *S. mutans*. Species such as *S. sanguis*, *A. viscosus* and *S. mutans* are plaque formers in the supragingival domain,[33] and the latter two species have been associated with root surface caries.[34] *A. viscosus* has been associated with gingivitis in the experimental gingivitis model[35] and possesses *in vitro* both mitogenic and antigenic activity.[36,37] Thus, the proportional ascendency of these species in nondisease-associated plaques would appear to contradict certain evidence implicating these organisms as odontopathogens.

Evidence of microbial behavior *in vitro* or in the supragingival domain, however, does not predict that microbe's behavior in another environment, such as the subgingival domain. It is possible that *S. sanguis* and *A. viscosus*, through the production of soluble factors such as hydrogen peroxide and bacteriocins, could reduce the plaque levels of presumably more virulent anaerobic organisms, such as the spirochetes. Also, the mitogenic and antigenic components of *S. sanguis*, *A. viscosus* and similar organisms could elicit the particular T lymphocyte response that is associated with a nonprogressive early lesion.[38,39] In this sense, these nondisease-associated organisms may prime the host T cell response in such a way that a tissue-destroying B cell infiltration[39–41] does not occur.

The four LJP patients were notable in not having detectable *A. actinomycetemcomitans*. While *A. actinomycetemcomitans* can be significantly associated with LJP,[42] cases have been described where it cannot be isolated.[43] The LJP patients had no detectable *B. gingivalis* but were colonized by *B. intermedius*. Several investigators have reported spirochetes as undetectable,[44] or low[9] in LJP patients. This heterogeneity was observed in the present patients, as in two patients no spirochetes could be detected (Table 5).

The bacterial profile of the subgingival plaques in the AP individuals was rather uniform in that plaques removed from the Types III and IV patients were bacteriologically comparable in having high proportions of spirochetes, moderate proportions of *B. inter-*

medius and low proportions of *B. gingivalis* and *Veillonella* (Tables 3 and 4). As the bacteriological sites were selected as the most diseased site per quadrant and had similar clinical morbidities (Table 2), the bacteriological findings probably reflect that the same type of infection was occurring in an active site in any AP patient. The quantification of the clinical morbidity, as is done by the ADA Types III and IV classification system did not seem to provide any diagnostic help in differentiating between types of infections within this large group of AP patients.

The spirochetes were the overwhelming microbial type in the plaques of our AP patients. This agrees with dark-field and electron-microscopic studies by Listgarten and his colleagues[4,44] and phase-contrast microscopic observations by Keyes and Rams,[45] which collectively make an impressive argument that spirochetes are pathognomonic in periodontal disease.[46] One spirochete, *Treponema denticola* possesses a trypsin-like enzyme which could be a virulence mechanism in the periodontal pocket[23] and produces a soluble factor which causes a dose-dependent inhibition of murine and human fibroblast proliferation.[47]

Against this background of a spirochetal infection, other plaque organisms, such as BPB, may occasionally overgrow, thereby causing perhaps a more "acute" infection. For example, in one investigation BPB were isolated from seven to eight plaques removed from eight patients aged 34 to 48 years at levels that averaged 32% of the cultivable flora.[11] Other investigators have associated *B. gingivalis* (*B. asaccharolyticus*) with deep pockets,[7] marked gingival inflammation[7] and ADA Types III and IV.[6] In these studies the age range of the patients was from 24 to 66 years, so that it is possible that some of these patients would have resembled our EOP patients. These studies reported that *B. gingivalis* was present in 40 to 55% of the examined pockets, a finding that was similar to that which was observed in the EOP patients (Table 6). *B. intermedius* was present in most individuals in both disease-associated and non-disease-associated plaques,[6,7] but it can also be found in high proportions in the absence of *B. gingivalis* in adults with moderate to severe bone breakdown.[48]

The patients designated as early onset periodontitis (EOP) differed from the other patients by their relative youth and by their significantly higher proportions of *B. gingivalis* and/or *B. intermedius*. Young adult patients with a similar clinical appearance have been described in the recent literature and have been given descriptive terms, such as rapidly progressive periodontitis,[28] severe periodontitis,[10] advanced destructive periodontitis,[14] postjuvenile periodontitis[49] and generalized juvenile periodontitis.[29] These epithets were deemed less descriptive than "early onset" for the following reasons:

The designation "rapidly progressive" requires that at least two clinical examinations be made over time in

order to document the rapid progression of periodontal destruction. Most patients with this periodontal condition are not likely to have been observed thusly, so that it is doubtful that many clinicians or researchers could validly make this diagnosis. The terms "severe" and "advanced destructive" are appropriate, descriptive terms of the clinical morbidity observed, but do not convey a sense of the patient's youth. "Post juvenile" and "generalized juvenile" do convey the young age of the patient but suggest that the condition is or may be a sequela of LJP, for which currently no solid evidence exists.

Early onset periodontitis was recently used in referring to the periodontal findings in seven siblings who had either rapidly progressive periodontitis or juvenile periodontitis and in whom *Bacteroides* species were prominent members of the cultivable plaque flora.[50] This descriptor seemed appropriate for our patients, as the designation "early onset" could represent both an early stage of periodontitis as seen in our Group A patients, as well as an early age of onset of periodontitis as seen in our Group B patients. EOP thus is a collective descriptor that can accommodate most, if not all, the periodontal conditions seen in young individuals that cannot be diagnosed as LJP.

That EOP represents more than one bacterial infection is amply demonstrated by the present findings and the reports of others.[8,10] Our Type A patients had primarily a *B. gingivalis* infection with moderate proportions of *B. intermedius* and spirochetes, high proportions of cocci and no detectable *A. actinomycetemcomitans*. Our Type B patients had very high proportions of spirochetes and either high proportions of *B. gingivalis* or *B. intermedius* and no detectable *A. actinomycetemcomitans*. Tanner et al.[8] isolated high proportions of *B. gingivalis* from four sites in two young individuals who exhibited "widespread destructive lesions involving most of the dentition." These sites also harbored high proportions of *A. actinomycetemcomitans* in their plaques. A third patient, who resembled the other two and our Type B patients, clinically had high proportions of *B. gingivalis* but no detectable *A. actinomycetmcomitans*. Spirochetes were not monitored. Moore et al.[10] sampled 34 affected pocket sites in 21 patients whose clinical description resembled that of our Group B patients. They found a complex flora containing 146 bacterial taxa, but no *B. gingivalis*. Instead, *F. nucleatum*, *Eubacterium timidum*, *E. nodatum*, *Lactobacillus minutus*, *B. intermedius* and two treponemes were found in elevated proportions. *Eubacterium* and *Lactobacillus* species would not be identified by our protocol, but *F. nucleatum* was, and could not be associated with EOP (Tables 3 and 5).

Studies which monitor antibody titers in the peripheral blood against a panel of putative periodontal pathogens implicate *B. gingivalis* in EOP. Mouton et al.[12] found that 50% of the individuals in a generalized juvenile periodontitis group had very high levels of IgG antibodies to *B. gingivalis* (*B. asaccharolyticus*). Taubman et al.[13] reported that the level of serum IgG antibody to *B. gingivalis* was higher in generalized juvenile periodontitis patients than in adult periodontitis patients. Ebersole et al.[14] showed that 43% of the sera from a group of 62 patients categorized as advanced destructive periodontitis had high IgG antibodies to *B. gingivalis* and that a substantial proportion of these patients exhibited high antibody responses to both *B. gingivalis* and *B. intermedius*.

These bacteriological and immunological studies make a cogent argument for a role of *B. gingivalis* and possibly *B. intermedius* in some EOP patients. However, this role is clearly shared with, or may even be preempted in the Type B patient by the spirochetes. The spirochetes were present at levels greater than 40% of the flora in 13 of 14 Type B patients and averaged 30% in the other patient. The universality and magnitude of this spirochetal infection would make it the common bacterial denominator in Type B patients and would make these patients bacteriologically similar to the AP patients. Other investigators have reported that spirochetes averaged 56% of the flora in plaques removed from 8 post juvenile periodontitis patients,[49] and 57% of the flora in plaques removed from 8 mm or deeper pockets in 22 patients between the ages of 21 and 28.[30] Thus, spirochetes, and not BPB or *A. actinomycetemcomitans*, are the organisms most typical of the Type B, EOP patient.

If this is so, then the recognition of the Type A patients is of some interest, as in these patients the *B. gingivalis*-*B. intermedius* infection was comparable to the spirochetal infection in terms of microbial numbers and frequency of occurrence (Tables 4–7). The EOP Type A patients were initially classified as ADA Type II patients on the basis of probing depths and clinical attachment loss (Table 1), but because of the bacteriological findings were placed into the EOP category on the assumption that the Type A patient represented an early stage of a periodontal infection, involving primarily BPB, that occurred prior to a spirochetal overgrowth. It would appear from examining the other disease categories that as the spirochetes became the dominant entity in the plaque flora, the pockets were deeper (Table 2), and that the incidence of a detectable *B. gingivalis* colonization declined both in the EOP Type B and AP Types III and IV patients (Tables 5 and 6). This suggests that the ecological niche occupied by *B. gingivalis* is taken over by the spirochetes or some other unidentified species, either by an active process, i.e., bacterial antagonism, or by environmental conditions, i.e., low Eh, low pO_2[51] that favor the spirochetes or by host antibodies against *B. gingivalis*.[12–14]

Thus, a periodontopathic succession may exist in the subgingival plaques as *B. gingivalis* may be replaced by spirochetes as the periodontal condition progresses

from a Type A-EOP to a Type B-EOP and later, on to AP. Diagnosis and treatment of the Type A-EOP patient might interrupt this sequence. The occasional occurrence of *B. gingivalis* in high proportions in plaques taken from AP patients (Table 6) could coincide with the asynchronous multiple bursts of periodontal destruction that have been observed in untreated periodontal patients.[52,53] If this is so, then *B. gingivalis* could be the microbial indicator of acute infections whereas the spirochetes could be indicators of chronic infections.

The present study indicates that the various types of periodontitis, with the possible exception of LJP, are specific anaerobic infections involving spirochetes and to a lesser extent BPB. This could then explain the success of metronidazole, an antimicrobial that has a spectrum of activity limited to anaerobic bacteria, in the treatment of deep pockets highly infected with spirochetes.[5,32,54]

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Send reprint requests to: Dr. Walter J. Loesche, Professor of Dentistry, University of Michigan School of Dentistry, Ann Arbor, MI 48109.

ERRATUM

In his article entitled: Dentinal Sensation and Hypersensitivity, A Review of Mechanisms and Treatment Alternatives which appeared in the April 1985 issue of the *Journal of Periodontology*, Dr. Berman misidentified the dentifrices which were compared by Tarbet et al.

The product Protect, not Promise, was a test dentifrice in the cited Tarbet reference.