THE ROLE OF SPIROCHETES IN PERIODONTAL DISEASE

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Adv Dent Res 2(2):275–283, November, 1988

ABSTRACT

The spirochetal accumulation in subgingival plaque appears to be a function of the clinical severity of periodontal disease. It is not known how many different spirochetal species colonize the plaque, but based upon size alone, there are small, intermediate-sized, and large spirochetes. Four species of small spirochetes are cultivable, and of these, *T. denticola* has been shown to possess proteolytic and keratinolytic enzymes as well as factors or mechanisms which suppress lymphocyte blastogenesis and inhibit fibroblast and polymorphonuclear leukocyte (PMNL) function. All of these attributes could contribute to periodontal tissue insult. Yet independent of these potential virulence mechanisms, the overgrowth of spirochetes can be clinically useful if simply interpreted as indicating the result of tissue damage. In this case, the spirochetes would be indicators of disease and could be easily monitored by microscopic examination of plaque, or possibly by the measurement of benzoyl-DL-arginine-2-naphthylamide (BANA) hydrolytic activity in the plaque.

INTRODUCTION

The oral spirochetes are often the dominant bacterial types observed in subgingival plaque removed from diseased periodontal sites, and yet they are one of the least-studied and -understood members of the plaque flora. Their contributions to periodontal disease cannot be properly assessed until we know what species are present and what their metabolic and physiological characteristics are. This dearth of knowledge relative to the oral spirochetes reflects the difficulty that investigators have in isolating them from the plaque, and in maintaining them *in vitro*, once isolated. At the present time, we do not know how many different species of spirochetes can be cultivated; whether certain types such as the intermediate-sized and large spirochetes can be cultivated; what proportion of the total spirochetes is composed of the known cultivable species; or what factors produced by the spirochetes are harmful for the periodontium. Until these basic questions can be answered, there is no way to evaluate the role of spirochetes in periodontal disease, and accordingly, our understanding of the pathogenesis of periodontal disease remains suspect.

Presented at the Sunstar Portside Symposium, November 14-15, 1986, Kobe, Japan

This investigation was supported by grants DE 030211 and DE 06030 from the National Institute of Dental Research.

ACQUISITION OF ORAL SPIROCHETES

Definitive information on the acquisition of the oral spirochetes is lacking, forcing one to sketch in outline form what is known and/or presumed to be known. The human oral spirochetes appear to be distinct from human genital and intestinal spirochetes, from animal species, from overtly pathogenic species, and from free living forms (Canale-Parola, 1977; Harwood and Canale-Parola, 1984). This implies that the oral spirochetes are acquired from other humans *via* oral contact. We have detected spirochetes by dark-field microscopy in the dental plaque of about 40% of the 3- to 5-year-old children and in about 50% of the 6- to 12-year-old children whom we have examined. However, their numbers were less than 0.5% of the flora, and they were uncultivable. Almost all 6- to 12-year-old Dutch and Tanzanian children examined had detectable spirochetes in their plaque, and their numbers and proportions were greater when the plaque samples were removed from sites of gingival bleeding (Mikx *et al.*, 1986).

These data suggest that most if not all individuals acquire some type of spirochete in their early life. Since the oral spirochetes comprise at least four species, and undoubtedly more (Moore *et al.*, 1985), one

assumes from the above frequency data that the acquisition of any one of these spirochetes is a likely event once the teeth erupt. Once acquired, the spirochetes show a predilection for the subgingival plaque, presumably because in this ecosystem these motile organisms are not at as great a risk of being swept away by the saliva and masticatory forces. Also, the lower oxygen tension present in the subgingival plaque, combined with the availability of pre-formed nutrients derived from the host and cohabitant plaque bacteria, would contribute to their establishment in this site (Loesche, 1976; Loesche and Laughon, 1982).

ISOLATION

There are four species of oral spirochetes — *Treponema denticola, T. socranskii, T. vincentii,* and *T. pectinovorum* — which have been well-characterized, and American Type Culture strains are available (Table 1). Two other species, *T. macrodentium* and *T. oralis,* have been described in the older literature (Socransky *et al.,* 1969), but since type cultures are nonexistent, their relationship to the above species, and hence their uniqueness, has not been established. *T. denticola* and *T. vincentii* are asaccharolytic species, whereas *T. socranskii* ferments glucose and other sugars, and *T. pectinovorum* has a requirement for pectin (Smibert and Burmeister, 1983). No one has reported on the proportions of any single species such as *T. denticola, T. socranskii,* etc., in plaque associated with health or disease until 1988 (Simonson *et al.,* 1988). Rather, when spirochetes were isolated from the plaque, the data were always qualitative, *i.e.,* frequency of isolation (Smibert *et al.,* 1984), number of morphotypes isolated (Cheng and Chan, 1983; Fiehn and Westergaard, 1984), and so forth. This is because of the difficulty investigators have in the quantitative recovery of spirochetes from the plaque.

Spirochetes are delicate organisms relative to the other bacterial types that are found in dental plaque. This affects their isolation, since procedures used to disrupt the plaque — so as both to facilitate colony formation from single cells and to maximize the number of colonies — usually lyse the spirochetes. This was demonstrated by experiments in which subgingival plaque samples were gently disrupted by mechanical mixing (10 sec, Vortex), for a microscopic count to be obtained, and then subjected to vigorous disruption by either sonication or homogenization with a Tekmar homogenizer, or by being mechanically mixed for 60 sec (Vortex) (Salvador *et al.,*1987) (Table 2).

Spirochetes averaged about 55% of the microscopic count but were less than 1% of the viable count. Only small spirochetes were isolated, and these were essentially *T. denticola* and a few *T. socranskii* isolates. Sonication, which gave the highest viable recoveries for plaque bacteria, yielded the lowest percentage recovery of spirochetes. Homogenization yielded the highest percentage recovery of spirochetes and a respectable recovery of other bacteria. Vortex mixing, which is a very common method of dispersing plaque when paper points are used for plaque collection, yielded the lowest recovery of plaque bacteria, but was comparable with the homogenizer in the percentage recovery of spirochetes.

These data indicate that the routine dispersal procedures used in cultural studies severely discriminate against the recovery of spirochetes as they lyse the spirochetes, *i.e.,* spirochetes cannot be found by microscopic examination of the dispersed plaque. However, it is possible that some of the spirochetes might have survived dispersion, but did not grow because the medium was lacking in essential nutrients. This was not the case for *T. denticola, T. socranskii, T, vincentii,* and *T. pectinovorum,* since our medium could support their growth. Thus, the low or non-recovery of these species reflected either that they were present in appreciable numbers in the plaque and were lysed by the dispersal procedures, or that they were present only in low numbers in the plaque.

Most intermediate-sized and large spirochetes have probably never been cultivated. This could reflect the

TABLE 1
TAXONOMIC CHARACTERISTICS OF ORAL SPIROCHETES

Species	Size	No. of Axial Fibrils	Carbohydrate Fermentation	% G+C
T. denticola	small	2-4-2, 5-10-5	no	37-38
T. vincentii	intermediate	5-10-5	no	44
T. socranskii	small	1-2-1	yes	51
T. pectinovorum	small	2-4-2	pectin	39
T. oralis	small	1-2-1	no	?
T. macrodentium	small	1-2-1	yes	?

Based upon data which appeared in Loesche and Laughon (1982), Smibert and Burmeister (1983), and Smibert *et al.* (1984).

TABLE 2
EFFECT OF DISPERSAL PROCEDURES ON RECOVERY OF SPIROCHETES FROM SUBGINGIVAL PLAQUE SAMPLES (n=15)

Dispersal Procedure	Morphotype			
	small	intermediate	large	Total
	Spirochetes as % of Microscopic Count			
Gentle Mechanical	31%	15%	8%	54%
	Spirochetes as % of Viable Count			
Harsh Mechanical	0.8%	no growth	no growth	0.8
Homogenization	0.9%	"	"	0.9
Sonication	0.1%	"	"	0.1

[a]91% of isolates were *T. denticola*, 5% were *T. socranskii*, and 4% were unspeciated.
Adapted from Salvador *et al.*, 1987.

failure of the isolating medium to contain the specific nutrients which these organisms require, since even when gentle isolation procedures, *i.e.*, filter plate (Loesche and Socransky, 1962) and well plate (Loesche, 1976), are used under strictly anaerobic conditions, these organisms do not grow. A case in point is the requirement for pectin or its constituents, galacturonic and glucuronic acids, by *T. pectinovorum* (Smibert and Burmeister, 1983). If these unusual substrates were not present in the medium, then this species would not have been isolated from the plaque. This then requires the investigator to know *a priori* what the nutrient requirements are, or to "shot-gun" by using complex media that are supplemented with biological fluids such as rabbit serum, rumen fluid, or ascitic fluid. The latter approach has led to the isolation of *T. denticola, T. vincentii,* and *T. socranskii,* but information concerning the energy metabolism of these organisms is scant.

ASSOCIATION STUDIES

Despite these difficulties in the cultivation of spirochetes, there is abundant literature based upon microscopic examination of plaque samples to implicate spirochetes in periodontal disease. Spirochetes are usually not detected, or are present in low numbers and proportions in plaque removed fron non-diseased sites in children (Moore *et al.*, 1984; Mikx *et al.*, 1986) or in adults (Lindhe *et al.*, 1980; Loesche *et al.*, 1985; Theilade *et al.*, 1966). They increased in numbers and proportions in plaque associated with gingivitis (Lindhe *et al.*, 1980; Listgarten and Helldén, 1978; Listgarten and Lewis, 1967; Loesche *et al.*, 1982; Mikx *et al.*, 1986; Schultz-Haudt *et al.*, 1954) and reach their highest values in plaque removed from sites with periodontitis (Fig.1). Thus, spirochetes average 40% of the microscopic count in plaque removed from sites classified as adult periodontitis (AP) (range 19 to 57%, 16 citations) and average 50% of the flora in early-onset periodontitis (EOP) (Lindhe *et al.*, 1980; Loesche *et al.*, 1985). Only in localized juvenile periodontitis (LJP) are high proportions of spirochetes an inconsistent observation (Loesche *et al.*, 1985; Newman and Socransky, 1977; Slots, 1976). In none of these microscopic studies were data on the presence of any particular species provided, although the proportions of small, intermediate-sized and large spirochetes were often noted.

Cultural studies indicate that *T. denticola* is significantly associated with diseased sites (Moore *et al.*, 1985). Simonson *et al.* (1988) have used monoclonal antibodies to *T. denticola* to show that the absolute and relative numbers of this species increased significantly in plaque removed from diseased sites compared with plaque removed from non-diseased sites. We also have data obtained with monoclonal antibodies which associate *T. denticola* with clinical disease (Lopatin, Bretz, and Loesche, in preparation).

These association data indicate that spirochetes are dominant organisms in plaque removed from diseased sites. The number of studies that associate the spirochetes with periodontal disease is more than that for any other putative periodontopathogen. While the average proportions of spirochetes are appreciably higher than those reported for *Actinobacillus actinomycetemcomitans* (Kornman and Robertson, 1985; Slots and Genco, 1984; Slots *et al.*, 1985), *Bacteroides gingivalis* (Loesche *et al.*, 1985; Moore *et al.*, 1982a; Slots and Genco, 1984; Slots *et al.*, 1985), *Bacteroides forsythus* (Dzink *et al.*, 1985), *Eubacterium* species (Moore *et al.*, 1982b), and a host of lesser contenders, there is some reluctance on the part of the periodontal community to accept spirochetes as periodontopathogens. Some of this is the "chicken *vs.* the egg" argument, which sees the spirochetes as opportunistic organisms thriving on the nutrients that presumably are relatively abundant in a periodontally inflamed site. However, this argument applies equally well to the other putative periodontopathogens, for which there are also mainly association data (Dzink *et al.*, 1985; Loesche *et al.*, 1983; Newman and Socransky, 1977; Slots, 1976; Slots and Genco, 1984; Slots *et al.*, 1985).

Only a few studies have compared the proportions of *B. gingivalis* and spirochetes in the same plaque samples. The most extensive of these is one in which 423 subgingival plaque samples were taken from the most "diseased" site *per* quadrant in each of 112 patients (Loesche *et al.*, 1985). The findings showed that very high proportions of spirochetes, *i.e.*, >40%, were characteristic of plaque removed from patients diagnosed as having either adult periodontitis (AP) or early-onset periodontitis (EOP) (Figs. 2a and 3a). In those patients diagnosed as exhibiting adult periodontitis, there was no plaque in which spirochetes were not detected (ND), and 70 to 80% of the patients had plaque which averaged more than 40% spiro-

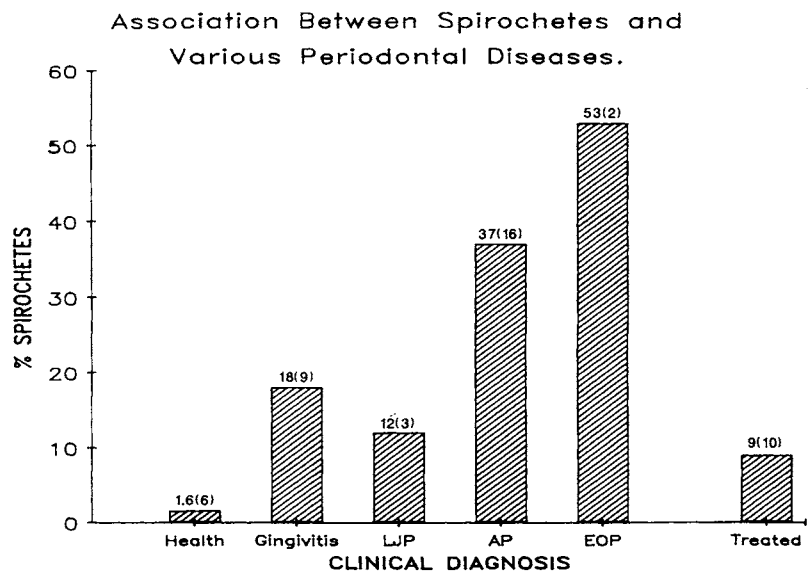

Fig. 1 – LJP is localized juvenile periodontitis; AP is adult periodontitis; EOP is early-onset periodontitis; treated represents the spirochete levels found in subgingival plaque of patients who have received either mechanical debridement and/or chemical antimicrobial treatments. Height of bar reflects the average percentage of spirochetes found in plaque taken from individuals with the cited clinical diagnosis. The number in parenthesis is the number of separate studies that were used to calculate the average value, *i.e.*, for AP 16 studies were reviewed in which the average value for spirochetes was 37%.

chetes (Fig. 2a). A similar spirochetal infestation was found in plaque obtained from patients diagnosed as having early-onset periodontitis (Fig. 3a).

In contrast, *B. gingivalis* could not be detected in the plaque of the majority of patients diagnosed as having adult periodontitis (Fig. 2b) and in some patients diagnosed as having early-onset periodontitis (Fig. 3b). However, *B. gingivalis* was prominent in many plaque samples taken from the early-onset periodontitis patients, often averaging about 10 to 20% of the cultivable flora. (The cultivable count is a fraction of the microscopic count and would be a smaller fraction, as the number of uncultivable spirochetes increases. Thus, in plaque with 50% spirochetes, a cultivable count of 20% *B. gingivalis* would be only 10% of the microscopic count.) *A. actinomycetemcomitans* was not detected by cultural means in any of these plaque samples.

These data would indicate that an increase in spirochetes is the most consistent microbiological finding in subgingival plaque taken from patients who have been diagnosed as requiring periodontal treatment because of existing periodontitis. This does not preclude a role for *B. gingivalis* and *A. actinomycetemcomitans* in some forms of periodontitis, since *B. gingivalis* was quite evident in most of the severe clinical cases (Fig. 3b), and *A. actinomycetemcomitans'* presence in severe forms of periodontal disease, especially localized juvenile periodontitis, is well-documented (Slots and Genco, 1984; Slots *et al.*, 1985). Rather, the data suggest that elevated levels and/or proportions of spirochetes are the most consistently found bacteriological indicator of periodontal disease, with the possible exception of those cases diagnosed by clinical means as localized juvenile periodontitis.

In certain clinical entities, spirochetes can overgrow in the plaque without being associated with the bone and attachment loss that is characteristic of periodontitis. This has been demonstrated for acute necrotizing ulcerative gingivitis (ANUG) (Listgarten and Lewis, 1967; Loesche *et al.*, 1982; Wirthlin and Devine, 1978) and may occur in other forms of gingivitis. For instance, a spirochetal-associated severe gingivitis has been described among certain South African Blacks which, based upon cross-sectional data, did not seem to progress to a periodontitis (Africa *et al.*, 1985). This was interpreted as indicating that spirochetes *per se* do not cause periodontitis. However, the spirochetes which overgrew in the gingivitis plaque, and which were associated with a severe inflammatory response, may not have included the same species of spirochetes which would be present in plaque associated with periodontitis.

This situation illustrates how important it is to be able to identify the various species within the spirochetal community in order for etiological relationships to be demonstrated. The analogous situation existed a few years ago with regard to the role of black-pigmented *Bacteroides* in periodontal disease. It was not until this group of organisms was separated into nine species (Coykendall *et al.*, 1980; Finegold and Barnes, 1977; Van Steenbergen *et al.*, 1984), that a clear role for *B. gingivalis* in some forms of periodontal disease was suggested (Loesche *et al.*, 1985; Slots and Genco, 1984). In order for this problem regarding the spirochetes to be addressed, it will be necessary for highly specific polyclonal and monoclonal antibodies to be prepared to all the cultivable spirochetes, not just *T. denticola*, and for these reagents to be used to obtain information relative to the proportions of the various cultivable small spirochetes in the plaque.

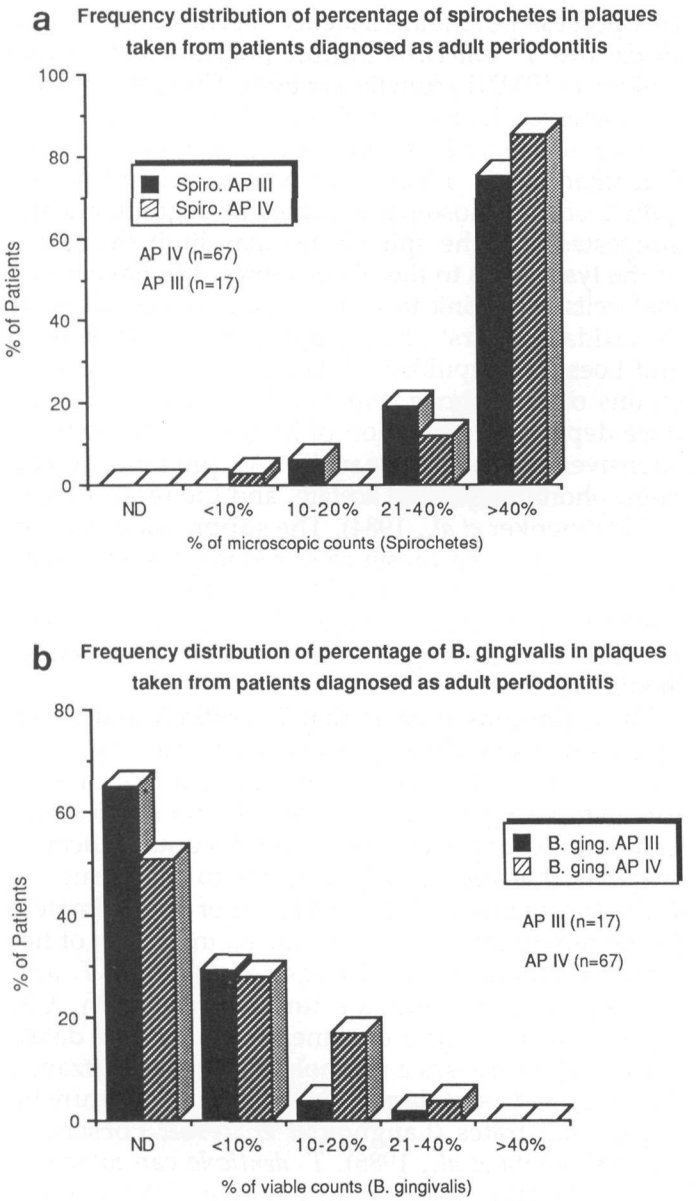

Fig. 2— (a) Frequency distribution of percentage of spirochetes in plaque taken from patients diagnosed as having adult periodontitis (AP). (b) Frequency distribution of percentage of *B. gingivalis* in plaque taken from patients diagnosed as having AP. Information taken from Loesche *et al.*, 1985.

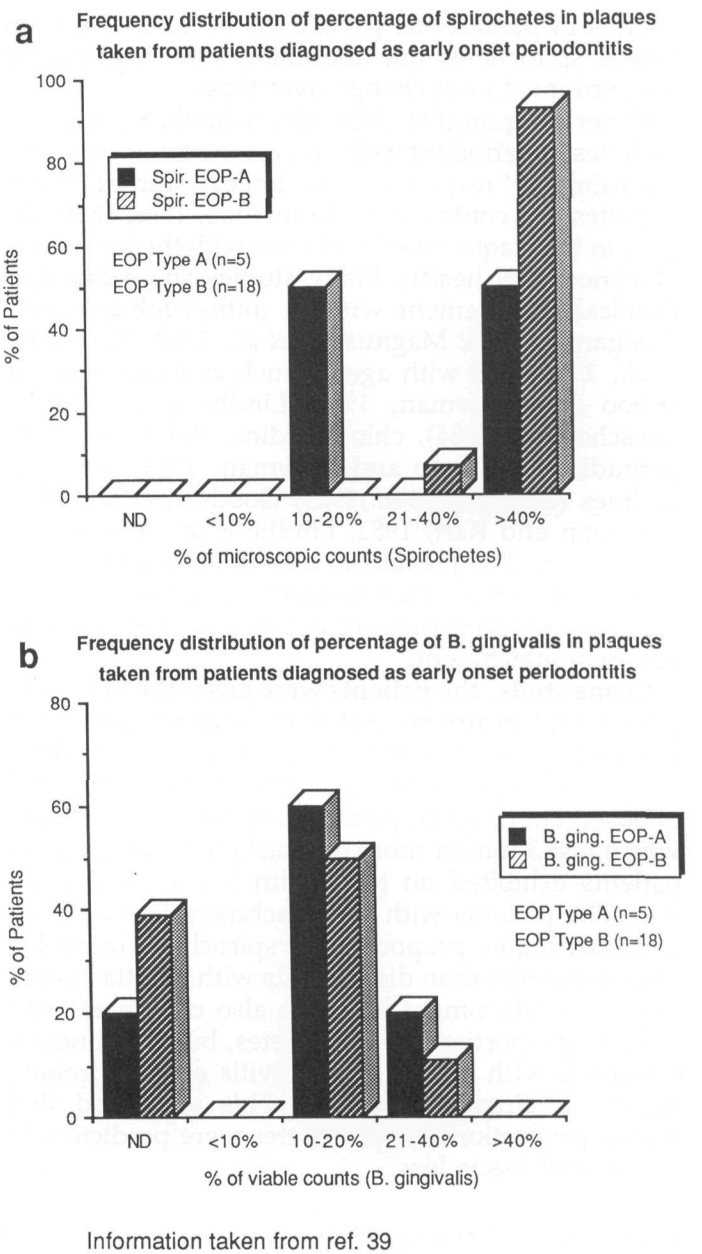

Fig. 3— (a) Frequency distribution of percentage of spirochetes in plaque taken from patients diagnosed as having early-onset periodontitis (EOP). (b) Frequency distribution of percentage of *B. gingivalis* in plaque taken from patients diagnosed as having EOP. Information taken from Loesche *et al.*, 1985.

LONGITUDINAL STUDIES

The progression of periodontal disease from gingivitis to periodontitis has not been documented in humans. Thus, there is no evidence on the shift in the flora that may accompany and/or cause this deterioration. There is one longitudinal study involving diseased sites that lost 2 mm of attachment during a single two-month interval (Dunham *et al.*, 1985). The spirochetes were numerically impressive members of the plaque flora before and after the attachment loss, so that their participation in the observed tissue destruction could not be eliminated. However, the lack of a discernible shift in the spirochetal proportions led the authors to conclude that spirochetes were not uniquely involved. Yet, shifts in proportions of bacterial species of a similar morphology cannot be detected by microscopic means. Thus, an increase in the proportions of a putative periodontopathogen, such as *T. denticola,* could have occurred and would not have been observed, if this increase were balanced by a decline in other species of small spirochetes. This underscores the need for identification and quantitation of species within the spirochetal community, so as not to dismiss the involvement of a specific

species in periodontal pathology, in those instances where spirochetes are abundant, but their overall proportions do not change over time.

Other longitudinal data which implicate the spirochetes in periodontal disease can be grouped under the rubric of "response to treatment" studies. If spirochetes are contributory to disease, then their demise in the plaque should coincide with the restoration of periodontal health. Many studies show that mechanical debridement without antimicrobial agents (Listgarten, 1984; Magnusson *et al.*, 1984; Mousques *et al.*, 1980) and with agents such as metronidazole (Khoo and Newman, 1983; Lindhe *et al.*, 1983b; Loesche *et al.*,1984), chlorhexidine (Addy and Langeroudi, 1984; Khoo and Newman, 1983), or tetracyclines (Goodson *et al.*,1979; Goodson *et al.*, 1985; Kornman and Karl, 1982; Lindhe *et al.*, 1983a; Listgarten *et al.*, 1978) results in a decrease in spirochetes and a concurrent improvement in health, as measured by reduced probing depth and/or increase in apparent attachment.

In one study, the patients were given the necessary periodontal treatment and then were not given any maintenance treatment during the recall visits (Listgarten and Levin, 1981). During the one-year period of observation, seven patients had two or more teeth which lost 3 mm or more of attachment, whereas six patients exhibited no teeth with 3 mm attachment loss. The patients with the attachment loss had significantly higher proportions of spirochetes in pooled plaque samples than did patients with no attachment loss. This attachment loss was also correlated with baseline proportions of spirochetes, but could not be correlated with plaque or gingivitis scores, probing depth, or gingival recession. This suggested that plaque proportions of spirochetes were predictors of subsequent tissue loss.

VIRULENCE MECHANISMS

If spirochetal overgrowth is synonymous with the presence of clinical inflammation, then it is likely that these organisms are active contributors to this inflammation. Data on how this is accomplished are minimal due to the aforementioned difficulty in isolating and growing many of these organisms. Spirochetes contain endotoxin and, therefore, would contribute to those toxic and pharmacological effects attributed to the endotoxin found in the subgingival plaque. They also possess a wide variety of proteolytic enzymes, including a collagenolytic enzyme (Makinen *et al.*, 1986). But more importantly from a virulence perspective, the small size of the spirochetes and their motility enable some of them to invade the periodontal tissue (Frank, 1980; Saglie *et al.*, 1982), where they could release their endotoxin and other toxic components directly adjacent to fibroblasts, epithelial cells, and other tissue components.

Recent studies have shown that the cultivable species possess potential virulence mechanisms. *T. denticola* and *T. vincentii* inhibit polymorphonuclear leukocyte (PMNL) function *in vitro*. Thus, the PMNLs were able to phagocytize these spirochetes, but were unable to degrade them (Boehringer *et al.*, 1986; Taichman *et al.*, 1982). This was associated with a failure of the lysosomal granules to degranulate and suggested that the spirochetes may limit the fusion of the lysosomes to the phagosomes. We have found that cells and sonicates of *T. denticola* can abrogate the oxidative burst of neutrophils *in vitro* (Robinson and Loesche, unpublished data). Sonicates of certain strains of *T. denticola*, but not *T. vincentii*, caused a dose-dependent inhibition of human lymphocyte responsiveness to concanavalin A, phytohemagglutinin, phorbol myristate acetate, and the recall antigen SKSD (Shenker *et al.*, 1984). The suppression was dependent upon the presence of monocytes and could be reversed or prevented by the addition of indomethacin and catalase, suggesting that both prostaglandins and hydrogen peroxide were involved as mediators.

These findings suggest that *T. denticola* and possibly the other spirochetes possess mechanisms by which they can evade the normal host surveillance that occurs in the subgingival ecosystem. Other studies suggest that these spirochetes can produce substances or enzymes that are directly harmful to the tissue. *T. denticola* contains a cell-bound factor of approximately 50,000 molecular weight that caused inhibition of fibroblast proliferation (Boehringer *et al.*, 1984). *T. denticola*, *T. vincentii*, and the unspeciated strain, US, possess collagenolytic enzymes (unpublished data). *T. denticola* possesses a fibrinolytic enzyme (Nitzan *et al.*, 1978) and peptidases which hydrolyze synthetic trypsin substrates (Laughon *et al.*, 1982; Loesche *et al.*, 1987; Ohta *et al.*, 1986). *T. denticola* can attach to epithelial cells in tissue culture (Olsen, 1984) and exhibits keratinolytic activity (Mikx and de Jong, 1987).

If any of these factors and enzymes were released in the subgingival ecosystem in ways that would bring them into contact with the host cells and tissue, then these spirochetal products could contribute to, if not account for, the histopathological features of periodontitis. The possibility of this contact occurring is amplified by the ability of the spirochetes to penetrate the epithelial spaces and below the epithelium into the connective tissue (Frank, 1980; Saglie *et al.*, 1982), and by the ability, at least of *T. denticola*, to inhibit PMNL function (Boehringer *et al.*, 1986; Taichman *et al.*, 1982).

The ability of the spirochetes to invade the host would imply that the host's immunological cells would be well-acquainted with spirochetal antigens. However, the few immunological studies measuring antispirochetal antibodies have not been helpful in associating spirochetes with disease. Steinberg (1970) reported high titers of circulating antibodies to spirochetes in individuals with moderate periodontitis but not in individuals with advanced disease. Man-

gan *et al.* (1982) found significant antibody titers to *T. denticola, T. vincentii*, and *T. phagedenis* in the sera of both healthy and periodontally involved patients. In another study (Tew *et al.*, 1985), those individuals with the most severe periodontal involvement exhibited a lower serological response to *T. denticola* and *T. socranskii* compared with healthy controls and with individuals with less periodontal morbidity. These findings demonstrate the immunogenicity of the spirochetes, but apparently did not utilize antigens that recognized a "diagnostic" antibody response. Alternately, they raise the possibility that at high antigen in the pocket ecosystem, as would occur in AP and EOP (Fig. 1), the spirochetes may cause some type of immune suppression which enables them to escape the normal immunologic surveillance of the host.

Diagnostic Implications of Spirochetes

The association and response to treatment studies indicate that subgingival plaque levels and/or proportions of spirochetes could be used clinically to identify those sites and/or individuals who require periodontal treatment. This information can be routinely obtained by the use of phase or dark-field microscopy using a simple qualitative (Keyes and Rams, 1983) or quantitative (Khoo and Newman, 1983) enumeration system. However, if other periodontopathic organisms are also involved in the plaque infection with the spirochetes, then these other organisms would not be detected by the microscopic approach, and accordingly some false-negative results would be recorded. Thus, a more broad-based diagnostic procedure would be preferred.

The taxonomic screening of periodontopathic organisms with various enzyme assays has shown that *T. denticola, B. gingivalis, B. forsythus,* and an unspeciated *Capnocytophaga* possess a trypsin-like enzyme that can be detected by the hydrolysis of benzoyl-DL-arginine-2-naphthylamide (BANA) (Loesche, 1986). This BANA hydrolytic enzyme could also be detected in subgingival plaque samples and was statistically related to the plaque levels and proportions of spirochetes and to probing depth (Loesche *et al.*,1987). Thus, a BANA-positive plaque was indicative of subgingival plaque containing more than 30% spirochetes that was removed from sites with probing depths of 7 mm or more. The other BANA-positive species accounted for only 10% of the BANA-positive reactions, indicating that the plaque BANA test was essentially a *T. denticola* reaction.

The relationship between BANA-positive plaque and spirochetes in over 400 subgingival plaque samples is shown in Fig. 4. Since both the number of spirochetes in a plaque sample, *i.e.*, spirochetes *per* high-power microscopic field (hpf), and the proportions of spirochetes increased, the proportions of BANA-positive plaque increased. When the spirochetes could not be detected, less than 10% of these plaque samples were BANA-positive, and these were mainly weakly-positive. At spirochete levels greater than two spirochetes *per* hpf, the majority of the samples were BANA-positive, and strong reactions greatly outnumbered weak reactions (Fig. 4).

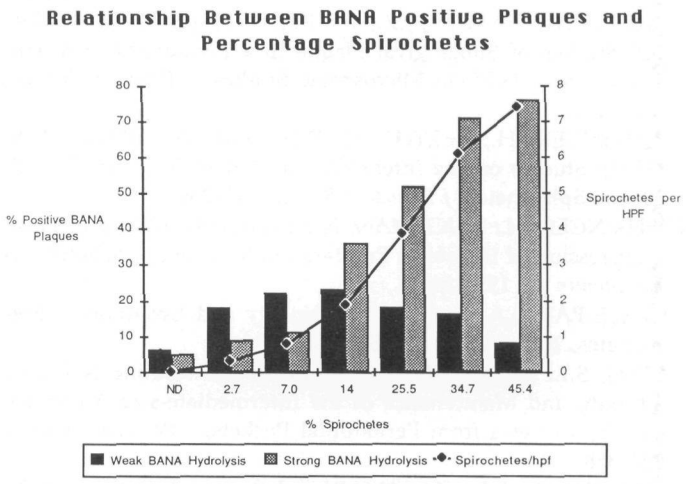

Fig. 4—Relationship between BANA-positive plaque and percentage spirochetes. Information taken from Loesche *et al.*, 1987.

Seventy-one percent of over 200 plaque samples removed from untreated periodontal patients were BANA-positive, averaged 40% spirochetes, and came from sites that had average probing depths of 7 mm. The BANA-negative samples from these patients averaged 12% spirochetes and came from sites that had average probing depths of 5.4 mm. In contrast, only 8% of 150 plaque samples removed from treated patients seen at recall visits were BANA-positive, and these had significantly higher proportions of spirochetes and came from deeper pockets than did the BANA-negative samples (Loesche *et al.*, 1987).

These data indicate that the ability of subgingival plaque to hydrolyze BANA is a reliable marker for the presence of high proportions of spirochetes and could possibly be used clinically to identify those sites and/or individuals who might require either initial treatment or re-treatment for reduction of this spirochetal overgrowth. If so, then BANA hydrolysis has the potential to be an objective indicator of periodontal disease and could be used in combination with clinical criteria, both for initiation of therapy and as a means to monitor the efficacy of treatment.

Acknowledgments

Constance and Dennis Lopatin assisted in the preparation of the manuscript.

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