

Presence of *Treponema denticola* and *Porphyromonas gingivalis* in Children Correlated with Periodontal Disease of Their Parents

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Abstract. Considerable evidence exists suggesting that periodontal disease is due to the overgrowth of a finite number of specific bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Treponema denticola*, *Bacteroides forsythus*, and *Prevotella intermedia*, among others. Three of these organisms—*P. gingivalis*, *T. denticola*, and *B. forsythus*—can be easily detected in plaque samples by the hydrolysis of the synthetic trypsin substrate benzoyl-DL-arginine-naphthylamide (BANA). The aim of the present study was to determine if a relationship could be found between the presence of either these organisms or periodontitis in the parent and the presence of BANA-positive species in the child. Thirty-four mothers or fathers and 34 children were examined for plaque scores, papillary bleeding scores, and the presence of *P. gingivalis* and *T. denticola* in four subgingival or marginal gingival plaque samples as assayed by the BANA test or specific polyclonal antibodies using an ELISA. Children whose parents were colonized by BANA-positive bacteria were 9.8 times more likely to be colonized by these BANA-positive species. Children whose parents had clinical evidence of periodontitis were 12 times more likely to be colonized by these BANA-positive species. These data are compatible with the hypothesis that children may acquire the BANA-positive species from their parents, especially if the parent has periodontitis.

Key words. Children, Diagnostic Tests, Periodontal Diseases, *Porphyromonas gingivalis*, *Treponema denticola*.

Introduction

Periodontal disease, like dental decay, appears to result from the absolute and relative increase of certain bacterial species in the subgingival flora (Loesche, 1987; Moore, 1987). Studies among mother-child pairs have suggested vertical transmission of *Streptococcus mutans* within human populations (Caufield *et al.*, 1988, 1993). The putative etiologic agents of periodontal disease may also be transmitted from parents, especially the mother, during childhood. For example, when a child was positive for *A. actinomycetemcomitans*, either the mother or the father was often positive for this organism (Alaluusua *et al.*, 1991).

Previously we had found that children whose parents had a documented history of periodontal disease had plaque which was more likely to be colonized by bacterial species capable of hydrolyzing the synthetic trypsin substrate benzoyl-DL-arginine-naphthylamide (BANA) than did children of parents with unknown periodontal status (Watson *et al.*, 1991). There are very few BANA-positive species among the cultivable plaque flora (Loesche *et al.*, 1990a). The three species always positive for BANA hydrolysis are *Treponema denticola*, *Porphyromonas gingivalis*, and *Bacteroides forsythus*. Since these species are frequently cited as periodontal pathogens, the BANA test has the potential for being a simple procedure for detecting these species in plaque samples. Subsequently, the BANA test was found to be comparable with DNA probes and immunological reagents in its ability to detect these three species in subgingival plaque samples from diseased periodontal sites (Loesche *et al.*, 1992).

In the present investigation, we used the BANA test to search for BANA-positive species in parent-child pairs. In

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addition, we were able to show a strong correlation between the parent's periodontal status and the presence of BANA-positive species in plaque samples taken from the children.

Materials and methods

Subjects

All parents of the 157 children seen in a previous study (Watson *et al.*, 1991) were invited by telephone to participate in this follow-up study. Only 34 parents (28 mothers and six fathers) agreed to participate. They represented 28% of the 125 families studied originally. Fifty-two children were studied—48 from the previous study plus four additional siblings. The types of data obtained previously for each child—i.e., birth date, gender, ethnic origin, history of periodontal disease in the household, and the child's usage of antibiotics—were also obtained for each parent.

Clinical examination

Each parent was given a thorough clinical examination in which probing depths and attachment levels were recorded by a periodontist at 6 sites for each tooth present. Additional measurements were taken in the following order: Plaque Index (PI) (Silness and L  e, 1964); collection of subgingival plaque samples; and Papillary Bleeding Scores (PBS) (Loesche, 1979). Other parameters noted were: tooth mobility, furcation involvement, number of teeth missing, and usage of partial (fixed or removable) prosthesis.

The examining clinician, who was unaware of the BANA status of either the children or the parent, used the above data to classify the patient as either periodontally healthy or having moderate or an advanced form of periodontitis, according to the American Academy of Periodontology Classification scheme (AAP, 1987).

Plaque sample collection

The tip of a sterile periodontal probe was used to obtain the subgingival plaque samples. First, the supragingival plaque was removed from the site by means of a curette and discarded. Then, the periodontal probe was introduced subgingivally between the first molar and second premolar of each quadrant, or, if one of these teeth was missing, then between either the first and second molars, or between the premolars.

BANA assay

The BANA reagent card (PerioScanTM, Oral-B Laboratories Inc., Redwood City, CA) was used. The tip of the periodontal probe containing the plaque was wiped onto the BANA-impregnated lower strip, located on the bottom of the BANA reagent card. The reaction was immediately activated by water applied to the upper strip containing the

fast black dye. The lower strip was folded onto the upper, and both strips were held in place with a metallic clip (Loesche *et al.*, 1990b). The card was placed in an incubator at 55°C for 15 min. Positive results appeared as blue spots that reflected weak or strong BANA reactions, whereas negative results did not show any color change.

ELISA assay

An enzyme-linked immunosorbant assay (ELISA) was used to detect *T. denticola* or *P. gingivalis* in the plaque samples that had been placed on the lower strip (Watson *et al.*, 1991). (An antibody to *B. forsythus* was not available at the time of this investigation.) Briefly, the lower strip was cut horizontally so that each half contained a portion of the plaque. Each half-strip was treated with immunological reagents highly specific for *T. denticola* or *P. gingivalis* by procedures described by van Poperin and Lopatin (1991).

Statistical analysis

Site-specific analysis was performed, accounting for the within-patient dependency of sites (Huj  el *et al.*, 1990). A beta binomial ANOVA model was used to correlate the within-patient (child) frequency of positive BANA or positive ELISA reactions to the (parent's) AAP classification of periodontal disease (generalized linear interacting model: GLIM). A Fisher Exact test was used whenever there were fewer than five observations in a cell.

Results

The demographic characteristics of the parent-child pairs are shown in Table 1. Within the child population, there were no significant differences among the measured parameters that related to gender, so all children were treated in the data analysis as a single population. Because there were only six fathers, the parent population was also combined as a single population with regard to gender. There were too few subjects in each racial/ethnic group for meaningful comparisons to be made, so this aspect of the study population was not statistically analyzed. However, 55% of the plaque samples in the African-American children were colonized by BANA-positive species, compared with 37% of the plaque samples of the non-African-American children.

Seventy percent of the 34 children tested positive and/or weak-positive for the presence of BANA-positive organisms in one or more of the four plaque samples. Eighty-eight percent of the 34 parents tested positive and/or weak-positive for the presence of BANA-positive organisms in one or more of the four plaque samples. The ELISA assay showed that all BANA-positive samples were positive for either *P. gingivalis* or *T. denticola* or both.

If the parent was colonized by BANA-positive species, then the odds ratio that the child would be colonized was

Table 1. Demographic characteristics of parent/child pairs

	Parent (34)	Child (34)
Age (yrs)	26-41	6-11
Gender	28 F/8 M	13 F/21 M
Race		
White	20	20
Afro-American	8	8
Other	6	6

9.8. This ratio was not significant (Table 2). The Fisher Exact test was marginally significant at $p = 0.07$. If the parent had periodontitis (moderate and advanced periodontitis were grouped together), then she/he was 5.18 times more likely to have the BANA-positive species in one or more plaque samples (Table 3) (sensitivity = 95%, 19/20; specificity = 21%). Children of a parent with periodontitis were 12 times more likely to be colonized by a BANA-positive species, and this ratio was significant (Table 3).

Discussion

This study showed that children who had BANA-positive plaque in one or more of the 4 test sites were 12 times more likely to have a mother or a father with clinically recognizable periodontal disease. These parents were demonstrably colonized with the BANA-positive species, since 19 of 20 parents with periodontitis were found to have one or more BANA-positive samples. These data confirm and extend our previous observation that children with BANA-positive plaque were likely to have one or both parents with a documented history of periodontal disease (Watson et al., 1991).

The inference from this is that the periodontally diseased parent is the most likely source of the child's BANA-positive species. This is supported by the concurrent observation that parents who were colonized by the BANA-positive species were also likely to have children with BANA-positive species in their plaque (Table 2). However, this latter relationship was not as strong as the periodontal association, presumably because the periodontally involved parents would be shedding more BANA-positive species into their saliva than would parents who were simply colonized.

This suggests that the BANA-positive species, *T. denticola*, and *P. gingivalis*, and possibly *B. forsythus*, which was not specifically sought for, as well as the few variably and weakly BANA-positive *Capnocytophaga* and non-pigmented bacteroides species (Loesche et al., 1990a), are transferred vertically from a parent, most likely the mother, to the child. Additional evidence for the presence of these anaerobes in children comes from many cultural and/or microscopy studies (Friskien et al., 1987, 1990; Mackler and Crawford, 1973; Mikx et al., 1986; Van Oosten et al., 1988),

Table 2. Relationship between the presence of BANA-positive species in the plaque of parents and that in their children

Presence of BANA-positive Species—Parent	Presence of BANA-positive Species—Child	
	No	Yes
No	3	1
Yes	7	23
	10	24
		34

Fisher Exact probabilities, $p = 0.066$.

Odds ratio (OR) = 9.8.

95% Confidence Limits, $0.7 < OR < 293$.

and from the presence of antibodies specific to organisms such as *P. gingivalis* and *Prevotella intermedia* in the serum of young children (Robert and Mouton, 1989; Cole et al., 1991).

The above scenario for vertical transmission, while compatible with the present finding, was not shown by those authors. Indeed, the association data presented could reflect that the parent was infected by the child, or that they both were infected from a common or different sources. For vertical transmission to be shown, some method of identifying specific strains by ribotyping or genomic DNA fingerprinting in both parent and child is needed. Preliminary studies in this regard with *A. actinomycetemcomitans* (DiRienzo et al., 1990) and *P. intermedia* (Genco and Loos, 1991) indicate that this approach is possible.

The present finding indicates that periodontal disease in one of the parents is a risk indicator for a child becoming colonized by one or more of the BANA-positive species, such as *T. denticola*, *P. gingivalis*, and possibly *B. forsythus*. However, when and how these organisms are transferred is not known, though presumably it is via salivary exposure. Whether this early colonization is a risk factor for periodontal disease in the child remains to be determined. In a periodontally treated adult population, BANA-positive plaque samples were associated with a subsequent loss of attachment (Loesche et al., 1990c). Clearly, the BANA test can be used to identify those youngsters who become colonized with these putative periodontopathogens, but longitudinal studies are in order to determine whether such children become periodontally involved sooner than children whose plaque remains BANA-negative.

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Table 3. Relationship between the presence of periodontitis in the parent, and the presence of BANA-positive species in the child

Presence of BANA-positive Species	Presence of Periodontitis		
	No	Yes	
Parent			
No	3	1	4
Yes	<u>11</u>	<u>19</u>	<u>30</u>
	14	20	34

Fisher Exact test, $p = 0.28$.
Odds ratio (OR) = 5.18.

Child			
	No	Yes	
No	8	2	10
Yes	<u>6</u>	<u>18</u>	<u>24</u>
	14	20	34

Fisher Exact test, $p = 0.006$.
Odds ratio (OR) = 12.

95% Confidence Limits, $1.59 < OR < 114.7$.

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