THE RELATIONSHIPS BETWEEN STREPTOCOCCAL SPECIES AND PERIODONTAL PATHOGENIC BACTERIA IN HUMAN DENTAL PLAQUE

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Summary—The existence of antagonistic and commensal relationships between microorganisms was investigated. The predominant cultivable flora in 172 plaque samples from active and non-active sites in 32 human subjects with destructive periodontitis was determined. The presence of putative periodontopathic organisms (Bacteroides gingivalis, Bacteroides intermedius, Bacteroides forsythus, Wolinella recta, Actinobacillus actinomycetemcomitans, and Eikenella corrodens) in a site was correlated with the absence of certain viridans streptococci (Streptococcus sanguis, Streptococcus uberis and Streptococcus intermedius), and vice versa. A strong commensal relationship was found between B. gingivalis and Strep. intermedius. The second study involved 3 subjects with intractable periodontitis whose plaque harboured large numbers of one or more of these periodontopathic organisms. This plaque contained fewer organisms capable of inhibiting the growth of the periodontopathic strains in vitro when compared with a clinically-healthy control subject. Intermediate levels of inhibitors were found in plaque taken from non-active lesions. The majority of inhibitors in plaque from the healthy control were viridans streptococci. Hydrogen-peroxide production by these organisms appears to be the principal mechanism of growth inhibition for periodontopathic organisms. Bacterial interactions may thus be causally related to both periodontal health and disease.

INTRODUCTION

Plaque samples from healthy gingival sulci typically contain large numbers of viridans streptococci, including Streptococcus sanguis and Streptococcus uberis; Actinobacillus actinomycetemcomitans is numerically much rarer (Mandell and Socransky, 1981; Slots, Reynolds and Genco, 1980). The opposite is true of plaque from clinically-active disease sites in localized juvenile periodontitis (Haffajee et al., 1984; Mandell, 1984). In vitro, Strep. sanguis and Strep. uberis can kill A. actinomycetemcomitans by virtue of their ability to produce hydrogen peroxide (Hillman and Socransky, 1981, 1982). Hammond et al. (1984) showed that A. actinomycetemcomitans produces a bacteriocin that can kill Strep. sanguis. Thus, the reverse relationship between streptococcal species and A. actinomycetemcomitans in healthy and diseased sulci may reflect in part the ability of these organisms to kill each other.

In a complex ecosystem, such as the periodontal pocket, antagonistic and commensal relationships are to be expected. Many, possibly most, of these would have minimal impact on the health of the periodontium. However, such relationships may be causally related to the maintenance of health or the initiation and progression of disease, particularly when putative periodontal pathogens are involved. Our purpose was to survey the periodontal flora for antagonistic and commensal relationships, with particular attention to those that involve organisms presumed to be pathogenic.

MATERIALS AND METHODS

Organisms and media

Strep. uberis, strain KJ2, and Strep. sanguis type II, strain KJ3 were isolated from the subgingival plaque of a 32 year-old subject with a clinically-healthy periodontium. Strep. sanguis type I, strain KJ4, Strep. intermedius, strain KJ5, Strep. morbillorum, strain KJ6 and Strep. mutans, strain BHT-2 were obtained from the Forsyth Dental Center collection. These strains were maintained in 30 per cent glycerol stabs at -20°C. Suspect periodontopathic organisms were Fusobacterium nucleatum, Eikenella corrodens, Bacteroides gingivalis, Bacteroides intermedius, Wolinella recta, Bacteroides forsythus (the fusiform Bacteroides), A. actinomycetemcomitans, and Capnocytophaga spitsigena. Reference cultures of these organisms were obtained from the ATCC or, in certain instances, freshly isolated from human dental plaque. They were maintained by weekly transfer on chocolate agar plates incubated at 35°C in an anaerobic atmosphere and frozen at -70°C.

Active periodontitis

Subgingival plaque samples were obtained from 172 active and inactive lesions in 3 subjects with localized juvenile periodontitis and 29 subjects with active destructive periodontitis. These samples were analysed for their predominant cultivable microflora using described methods (Haffajee et al., 1984; Dzink et al., 1985). In brief, samples were anaerobically dispersed, diluted and plated on tryptose soy agar supplemented with 5 per cent sheep blood (BBL). After 5-7 days anaerobic incubation, 50 adjacent isolates were picked from high dilution plates, streaked until pure, and characterized by a semiautomated technique (Dzink, Smith and Socransky, 1984) and end-product analysis. The strain features were used to identify the isolates via a probabilistic computer program.

Intractable periodontitis subjects

Three patients were diagnosed at the Forsyth Periodontal Center as having intractable periodontitis.
because they failed to respond to repeated scaling, surgery, low- and high-dose tetracycline therapy, pocket irrigation, antibacterial mouth-rinses, metronidazole (1 subject) and local delivery of tetracycline (Goodson, Haffajee and Socransky, 1979). Subgingival plaque samples from active disease sites and control sites were analysed for their predominant cultivable flora. Strains of suspected pathogens were selected from each active site on the basis of their higher proportionate incidence when compared to inactive sites in the same subject. A second set of plaque samples were tested for organisms inhibitory to the growth of these strains. Fifty random colonies on primary blood plates were stabbed with sterile toothpicks and replicated to chocolate-agar plates with and without a lawn of the pathogenic strain. The plates were incubated micro-aerophilically (0.1 per cent oxygen) for 2–7 days and zones of growth inhibition recorded. Isolates that had inhibitory potential were purified and restested, and then identified to the species level by the methods described above.

**In vitro assay for growth inhibition**

Wells, 5 mm in diameter, were punched in fresh chocolate-agar plates containing catalase (0, 1000 or 10,000 U/ml) or heat-inactivated (100°C, 5 min) catalase. Confluent lawns of the suspected periodontopathic organisms were prepared as follows: cells were scraped from 3 to 5 day-old chocolate-agar plates and suspended in 0.1 M phosphate-buffered saline (pH 7) to give an O.D. 590 of 0.5. Plates were cross-streaked with 0.25 ml of the cell suspensions using sterile cotton swabs. The wells were then filled with 5 μl of an overnight Todd–Hewitt broth culture of KJ2, KJ3, KJ4, KJ5, KJ6 or BHT-2. The plates were incubated in a micro-aerophilic atmosphere (0.1

![Fig. 2. Quantitative measurement of the relationship between *Strep. sanguis* Type II and *B. forsythus*. The relative risk is defined as the proportion of sites where *B. forsythus* is found in the presence of *Strep. sanguis II* divided by the proportion of sites where *B. forsythus* is found in the absence of *Strep. sanguis II*.](image)

![Fig. 3. The relative risk of finding *A. actinomycetemcomitans* or *B. gingivalis* in a site is plotted in relation to the proportion of *Strep. sanguis* type II present in the site.](image)
per cent O₂) for 2–7 days. Zones of growth inhibition were measured directly on the plates.

RESULTS

Bacterial interactions in plaque from periodontitis patients

The predominant cultivable floras were analysed to determine if the presence or absence of a particular so-called effector organism in a site related to the presence or absence of the following test strains: A. actinomycetemcomitans, B. forsythus, E. corrodens, B. gingivalis, B. intermedius and W. recta. The data were initially used simply to plot the site-specific occurrence of effector and test strains and their relationship to each other (Fig. 1). There were two basic patterns: Strept. intermedius and B. gingivalis typify the pattern in which both organisms are as likely to be found together in a site as either organism alone. The second pattern exemplified by Strept. sanquis type II and B. forsythus, is an example of several relationships in which the presence of one organism appeared to exclude the other.

The extent of these inter-relationships were quantified by methods outlined in Fig. 2. For example, with Strept. sanquis as the effector strain, B. forsythus was present in 10 per cent of all sites where Strept. sanquis was not found. For samples where Strept. sanquis was present B. forsythus was detected in only 1 per cent. The relative risk of finding the test strain in the presence and absence of the effector strain was expressed as the ratio of these percentages.

The ability of an effector strain to exclude or promote the presence of a test strain may require a threshold level of the effector, so the above analysis was repeated in increments for progressively higher proportions of the effector strain. The higher the proportion of Strept. sanquis in a site, the smaller is the relative risk of also finding A. actinomycetemcomitans there (Fig. 3). On the other hand, the presence of Strept. sanquis in a site, regardless of its concentration, had a minimal effect on the presence of B. gingivalis.

Table 1 summarizes the results obtained when the six test strains were analysed for their interaction with other plaque isolates. Clear-cut antagonisms, where

![Fig. 4. Proportion of isolates in plaque able to inhibit the growth of B. intermedius in vitro. Samples were from active disease and control sites of two patients (A and B) with intractable periodontitis and from sites in a healthy control patient.](image-url)

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Subject</th>
<th>Inactive</th>
<th>Inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. forsythus</td>
<td>A</td>
<td>8 (16/200)</td>
<td>20 (30/150)</td>
</tr>
<tr>
<td>W. recta</td>
<td>A</td>
<td>0 (1/400)</td>
<td>3 (4/150)</td>
</tr>
<tr>
<td>B. gingivalis</td>
<td>B</td>
<td>11 (2/200)</td>
<td>16 (24/150)</td>
</tr>
<tr>
<td>B. intermedius</td>
<td>A, B</td>
<td>13 (34/400)</td>
<td>37 (55/150)</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>A, B</td>
<td>6 (24/150)</td>
<td>16 (24/150)</td>
</tr>
<tr>
<td>E. corrodens</td>
<td>A, B</td>
<td>8 (32/400)</td>
<td>18 (27/150)</td>
</tr>
<tr>
<td>C. spitzigens</td>
<td>C</td>
<td>25 (32/200)</td>
<td>28 (42/150)</td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td>C</td>
<td>10 (21/200)</td>
<td>22 (33/150)</td>
</tr>
</tbody>
</table>
the average risk of finding a test strain in a site is half as likely with the effector strain present as with it absent, are shown by double asterisks. Major commensalisms, where the average risk of finding the test strain is at least twice as likely with the effector present as with it absent, are shown by single asterisks. Most of the major interactions with the six test organisms involved viridans streptococci. Streptococcal species interacted with the various test strains with some specificity: Strep. morbillorum interacted positively with A. actinomycetemcomitans and B. forsythus; Strep. intermedius showed the opposite effect with these two test strains, and also interacted positively with B. gingivalis; Strep. sanguis types I and II and Strep. iberis all interacted antagonistically with 2-4 of the test strains. Of the six test strains, only B. gingivalis was not markedly inhibited by any of the effector species.

Antagonistic relationships were studied in a more direct fashion in the experiment with plaque samples from healthy periodontal sites in a control subject and from active disease sites in 3 patients with intractable periodontitis. The results obtained when B. intermedius was the test organism are typical (Fig. 4). Thirty-seven per cent (55 of 150) of randomly selected primary plaque isolates from the healthy subject were capable of inhibiting the growth of B. intermedius strain BS6. These inhibitors were predominantly Strep. sanguis, Strep. iberis and Strep. intermedius. Two of our 3 intractable patients harboured large numbers of B. intermedius; their plaque was analysed for its content of inhibitors to this organism. In plaque taken from clinically-active lesions, only 1 per cent of the primary isolates (3 of 400 for patient no. 1 and 4 of 400 for patient no. 2) inhibited the growth of B. intermedius strain BS6. None of these inhibitors was viridans streptococci. In plaque from non-active sites in these same patients, 13 per cent (27 of 200 in both cases) of the primary isolates were able to inhibit the growth of B. intermedius. Of four such inhibitors only one was a viridans streptococcus, namely Strep. sanguis.

The proportion of inhibitors in plaque from active and inactive sites in the intractable periodontitis patients and from a healthy control patient are presented in Table 2. In general, plaque from the control contained significantly higher proportions of inhibitors to the various test strains than did plaque from active sites in the intractable patients. The only exceptions occurred when B. gingivalis and F. nucleatum were the test strains. Plaque from non-active sites in intractable patients tended to show intermediate levels of inhibitors to the various test strains.

When modification of the stab technique was used to test the ability of Strep. sanguis types I and II, Strep. iberis, Strep. morbillorum, Strep. intermedius and Strep. mutans to inhibit the growth of the six test strains in vitro, both Strep. sanguis and iberis could inhibit the growth of all the test strains to a variable extent (Table 3). The other streptococcal species had no effect on the growth of the test strains, except for Strep. intermedius and Strep. mutans, which clearly enhanced the growth of W. recta and B. gingivalis. Incorporation of catalase at a concentration of 1000 U/ml completely eliminated the zones of inhibition. Heat inactivation of the catalase before its
incorporation into the medium allowed the formation of inhibitory zones.

DISCUSSION

These three studies provide an insight into how bacterial interactions may be causally related to periodontal health and disease. Certain viridans streptococci, by virtue of their ability to produce hydrogen peroxide, appear to promote periodontal health by keeping the numbers of potentially pathogenic organisms below the threshold level necessary to initiate disease. Detailed investigation of any complex microbial ecosystem is likely to reveal both positive and negative interactions between some components. Frequently, an interaction may be characterized by the production of bacteriocins or essential growth factors by one organism that affect the viability of another. Direct evidence that such factors affect the types and numbers of organisms that occur in an ecosystem may be wanting. Strong correlations between the numbers and distributions of effector and test strains, as found in this study suggest that a particular mechanism is operative at that site. However, other investigations, such as mutant analysis (Hillman, Johnson and Yaphe, 1984), are required to provide definitive evidence.

The beneficial effect of certain streptococcal species on periodontal health has also been found in experimental gingivitis (Best et al., 1985). Certain types of periodontal disease may therefore result from an ecological imbalance which arises from the following sequence of events: first, unknown factors promote the relative outgrowth of an organism such as *Actinomyces actinomycetemcomitans* which produces a factor inhibitory to the growth of certain streptococcal species; this results in a reduction of local hydrogen-peroxide production, which in turn permits the outgrowth of various periodontal pathogens.

If this hypothesis proves valid, it may be that periodontal therapy should aim not only to eliminate periodontal pathogens but also to encourage persistent colonization and growth of protective species.

" Arrest of the disease process would then be followed by the establishment of a microbial flora that is balanced in favour of health.

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REFERENCES


