Replication of Periodontal Pockets by Microbial Pathogens in the Absence of Supportive Therapy*

Jacob Shiloah and Mark R. Patters

This clinical study evaluated the reinfection incidence by Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), and Prevotella intermedia (Pi) in periodontal pockets following scaling and root planing (SRP) and intrapocket irrigation with antimicrobial agents in a patient population who did not receive supportive maintenance therapy. The number of target organisms was determined utilizing DNA probes. Forty-one (41) inflamed pockets ≥ 5 mm with attachment loss and containing at least one target species were selected in 6 adult patients. Following a baseline clinical and bacterial examination, all patients received thorough SRP. In addition, 1 to 2 teeth in each patient were randomly assigned to each of the following 4 treatment modalities: 1) control group, no irrigation; 2) saline group, irrigation with 2 cc of 0.85% saline; 3) tetracycline group, irrigation with 2 cc of aqueous tetracycline HCl, 50 mg/ml (5%); and 4) chlorhexidine group, irrigation with 2 cc, respectively. All selected sites were non-adjacent. No additional therapy was rendered during the entire 1-year observation period. Clinical parameters and microbial analyses were recorded again at 1 week, and 1, 3, 6, 9, and 12 months post-treatment. The effect of antimicrobial irrigation on the reinfection rate of sites by Aa, Pg, and Pi was compared with that of the control groups (1 and 2) by ANOVA. No statistically significant differences were observed among the irrigation treatment groups with regard to any of the clinical or bacterial parameters studied. Therefore, the 4 treatment groups were combined into a single group whereby the rate of bacterial repopulation following extensive scaling and root planing could be ascertained. The infection incidence of sites at baseline (of total sites), 1 week and 12 months (of sites originally infected at baseline) was 14/41, 3/14, and 7/14 for Aa; 33/41, 6/33, and 12/33 for Pg; and 37/41, 3/37, and 12/37 for Pi, respectively. Thus, half or fewer of the originally infected sites became reinfected at 12 months despite lack of maintenance therapy. The results suggest that 1) a single episode of pocket irrigation with antimicrobial agents following thorough scaling and root planing did not affect the rate of repopulation of periodontal pockets by the tested pathogens; 2) thorough scaling and root planing has a lasting suppressive effect on selected periodontal pathogens for the majority of sites in patients with adult periodontitis; 3) pre-operative probing depth, the amount of gingival fluid flow and the composition of the subgingival microflora may serve as predictors for reinfection in the absence of maintenance care; and 4) reinfection of the treated sites by Aa, Pg, and/or Pi may constitute a risk factor that diminishes the effect of therapy in the absence of supportive maintenance care. J Periodontal 1996;67:130–139.

Key Words: Periodontal diseases/prevention and control; periodontal diseases/microbiology; periodontal pockets/microbiology; Actinobacillus actinomycetemcomitans; Porphyromonas gingivalis; Prevotella intermedia; reinfection.

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One of the main goals in the treatment of periodontitis centers on the reversal of the subgingival microflora from a “pathogenic” to “nonpathogenic” state and prevention of repopulation of subgingival plaque by “pathogenic” bacteria. This may be accomplished for most patients by a regimen that includes mechanical removal of the subgingival plaque and calculus from the root surfaces, home care, and periodic maintenance therapy. Professional root debridement suppresses spirochetes and motile rods and highly suspected periodontal pathogens such as Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), and Prevotella intermedia (Pi), while effecting an increase in coccoid cells.1-10 These shifts in the subgingival microflora parallel an improvement in all clinical parameters of periodontitis. However, these positive microbial changes appear transient.4,5,8,10 To sustain these positive effects, scaling and root planing must be performed periodically during the maintenance phase of periodontal therapy.11-16 Unfortunately, the optimal recall interval when scaling and root planing should be carried out to maintain a healthy subgingival microflora is decided empirically and requires further research.2 One means to approach this problem is not to decide recall interval based on changes in clinical parameters,17,18 but to study the kinetics of repopulation of the pocket environment by pathogenic flora following therapy.19-22

Many short duration studies on recolonization of the clinical crown by supragingival plaque following prophylaxis have been performed.23 However, reports on the reestablishment of a highly pathogenic subgingival flora following root debridement in the absence of maintenance therapy over a long observation period are few.24

The general objectives of this clinical study were to determine the rate of repopulation of periodontal pockets by Aa, Pg, and Pi following thorough scaling and root planing, without maintenance therapy, over a 1-year observation period and to learn if intra-pocket irrigation with antimicrobial agents retards the rate of repopulation observed following scaling and root planing alone.

MATERIALS AND METHODS

The methodology of this study has been completely described in a recent publication.25 Briefly, the study included 6 adult patients, 3 whites and 3 blacks, 2 males and 4 females, age 33 to 65 years, mean age 49.5 years, with moderate or severe chronic adult periodontitis. An additional patient was originally enrolled in the study, but was subsequently dismissed from the study 6 months postoperatively because of antibiotic therapy for a urinary tract infection. The patients were free of any known systemic diseases and none of them had taken antibiotics within the last 2 months or used antimicrobial oral rinses. A consent form was signed by each patient after a thorough explanation of the nature of this study and the risks involved.

Site Selection

Forty-one (41) inflamed pockets (5 to 8 non-adjacent teeth per patient) with 5 mm probing depth or greater and with probing attachment loss were selected in these patients. The sites, which included 28 non-molars and 13 molars, were selected because each harbored more than 6,000 cells of one or more of the following pathogenic bacterial species: Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), and Prevotella intermedia (Pi).

Bacterial Sampling

Bacterial samples were obtained by inserting a single paper point to the bottom of the pocket for 10 seconds. The identification and enumeration of bacterial target species were accomplished through DNA probe technology. The minimum detectable level of organisms by this test is 6,000 cells, and the maximum detectable level is 600,000 cells. Bacterial samples were obtained preoperatively (baseline, 2 to 3 weeks pre-scaling); at 1 week post-treatment; and at 1, 3, 6, 9, and 12 months post-treatment.

Clinical Parameters

The following clinical measurements were obtained at baseline; at 1 week post-treatment; and at 1, 3, 6, 9, and 12 months post-treatment: plaque index26 gingival index,27 gingival fluid flow using a Periotron 6000 following a 10-second sample onto a filter paper strip,1 probing depth to the nearest millimeter from the free gingival margin using a calibrated Michigan O probe with Williams markings, and probing attachment level to the nearest millimeter from the cemento-enamel junction (CEJ) using the above probe. The clinical indices were measured by one investigator who was trained and calibrated before the study and was not familiar with the treatment rendered. These parameters were also used to safeguard the patients’ welfare during the study.

Therapy

The patients received thorough scaling and root planing by an experienced clinician. It took 4 to 7 hours to complete the procedure using local anesthesia, ultrasonic tips and Gracey curets. At the end of the last scaling appointment, the selected sites were randomly divided into 4 groups. Each patient had at least one site assigned into each of the following treatment groups: 1) control group (no irrigation); 2) saline group (intrapocket irrigation with 2 cc saline); 3) tetracycline group (intrapocket irrigation with 2 cc of 5% aqueous tetracycline hydrochloride); and

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1OmniGene, Cambridge, MA.
2Pro Flow, Inc., Amityville, NY.
3Lederle Laboratories, Pearl River, NJ.
4) 0.12% chlorhexidine* group (intrapocket irrigation with 2 cc). One episode of irrigation was performed at the end of the last scaling appointment. The irrigation was performed with an endodontic syringe with 23 gauge needle that had a special notched tip that was inserted to the bottom of the pocket.* Careful isolation and suctioning was used to prevent any spill over onto other teeth. At the end of the irrigation episode the follow-up phase of the study began. No additional periodontal or preventive (prophylactic) therapy was rendered throughout the 1-year postoperative observation period.

As described in the results, no statistically significant differences were observed among the irrigation treatment groups with regard to any of the clinical or bacterial parameters studied. Therefore, the four treatment groups were combined into a single group whereby the rate of bacterial repopulation following extensive scaling and root planing could be ascertained.

**Statistical Analysis**

All hypothesis testing in this study used the site as the unit of analysis. The mean and standard deviation for all the variables studied were computed for each examination period. Repeated measures analysis of variance (ANOVA) was performed on all parameters over the course of study to detect significant differences among treatment groups. If statistically significant differences were observed, post-hoc tests were conducted to detect the source of these differences. Student t-test and chi-square analyses were also done as described in the results.

**RESULTS**

**Clinical Effects of Treatment**

Repeated measures ANOVA indicated that no significant differences among the irrigation treatment groups were noted for any of the clinical parameters. However, all clinical parameters of disease significantly decreased in all treatment groups over the 1-year observation period (data not shown). When combined into a single treatment group as shown in Table 1, scaling and root planing resulted in a significant decrease in plaque (P < 0.0001), gingivitis (P < 0.0074), gingival fluid flow (P < 0.0022), probing depth (P < 0.0001), and probing attachment loss (P < 0.0001).

**Microbiologic Effects of Treatment**

Repeated measures ANOVA indicated that no significant differences among the irrigation treatment groups were noted for any of 3 target species (data not shown). Additional testing established that no significant differences were evident in the number of sites infected by each of the target species or the number of species infecting the sites with regard to the treatment groups (data not shown). Therefore, the 4 treatment groups were combined into a single group whereby the rate of bacterial repopulation following extensive scaling and root planing could be determined.

**Number of infected sites.** The number and percentage of the 41 total sites infected by each of the 3 target species are shown in Table 2. Aa was detected in 14/41 of the sites at baseline, at 2/41 of the sites at 1 week, 1 month, and 3 months, and at 7/41 at 12 months. Pi was the most prevalent species preoperatively (90% of the sites) and again at each subsequent examination after 1 week. Over-

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Table 1. Effect of Treatment on the Clinical Parameters of 41 Sites During the 1-Year Examination Period

<table>
<thead>
<tr>
<th>Examination</th>
<th>Plaque Index ± 1 SE</th>
<th>Gingival Index ± 1 SE</th>
<th>Gingival Fluid Flow ± 1 SE</th>
<th>Probing Depth ± 1 SE</th>
<th>Probing Attachment Loss ± 1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.29 ± 0.10</td>
<td>1.54 ± 0.11</td>
<td>362 ± 41</td>
<td>5.9 ± 0.3</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>1 week</td>
<td>0.24 ± 0.08</td>
<td>1.22 ± 0.10</td>
<td>210 ± 34</td>
<td>4.6 ± 0.2</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>1 month</td>
<td>0.39 ± 0.09</td>
<td>1.17 ± 0.09</td>
<td>192 ± 25</td>
<td>4.2 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>3 months</td>
<td>0.46 ± 0.09</td>
<td>1.22 ± 0.08</td>
<td>243 ± 32</td>
<td>4.3 ± 0.2</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>6 months</td>
<td>0.37 ± 0.08</td>
<td>1.20 ± 0.06</td>
<td>253 ± 30</td>
<td>4.3 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>9 months</td>
<td>0.44 ± 0.11</td>
<td>1.15 ± 0.07</td>
<td>233 ± 29</td>
<td>4.5 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>12 months</td>
<td>0.49 ± 0.12</td>
<td>1.32 ± 0.08</td>
<td>225 ± 28</td>
<td>4.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

Table 2. The Number of Sites and the Percentage of Sites Remaining Infected by Each of the 3 Target Species at Each Examination

<table>
<thead>
<tr>
<th>Species</th>
<th>Baseline</th>
<th>1 Week</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
<th>9 Months</th>
<th>12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa</td>
<td>14</td>
<td>2 (14.3)</td>
<td>2 (14.3)</td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
<td>4 (28.6)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>Pg</td>
<td>33</td>
<td>6 (18.2)</td>
<td>5 (15.2)</td>
<td>1 (3.0)</td>
<td>5 (15.2)</td>
<td>6 (18.2)</td>
<td>12 (36.4)</td>
</tr>
<tr>
<td>Pi</td>
<td>37</td>
<td>3 (8.1)</td>
<td>7 (18.9)</td>
<td>6 (16.2)</td>
<td>6 (16.2)</td>
<td>11 (29.7)</td>
<td>12 (32.4)</td>
</tr>
</tbody>
</table>

*Peridex, Procter and Gamble, Cincinnati, OH.
*Sherwood Medical, St. Louis, MO.
all, 41/41 sites (100%) were infected by 1 or more species at baseline, while 11/41 (27%) sites remained infected at 1 week. At 3, 6, and 12 months, 9 (22%), 13 (32%), and 17 (42%) sites were infected with one or more of the target species, respectively (Table 3, bottom row).

The number and percentage of 41 sites infected by 1, 2, or all 3 of the target species at each examination are shown in Table 3. Of infected sites, the majority (21/41, 51%) were infected by 2 species at baseline and by 1 species at all subsequent examinations. Although 11 sites (27%) were infected by the cluster of all 3 target species at baseline (8 of this cluster were identified in 8 sites in one patient, 2 in a second patient and 1 in a third patient), no site became or remained infected by 3 species at 3 months. However, at 12 months, 4 sites (10%) became reinfected by all 3 species. Overall, therapy resulted in reduction of infection below detectable levels by the 3 target species in 78% of the infected sites at 3 months, 68% of the sites at 6 months, and in 58% of the sites at 12 months.

Table 4 illustrates the effect of therapy on the infection pattern of sites that were originally infected with 1, 2, or 3 of the target species. Of the 9 original sites infected with 1 species, only 2 sites remained infected at 6 and 12 months and none of them became infected with all 3 species over the course of the study. Two of the target species were harbored in 21 sites at baseline, but were reduced below detectable levels at the majority of the sites at 1 month (4 sites had 1 species and 2 sites had 2 species). However, of the original 21 sites infected by 2 species at baseline, 2 sites became infected by the third species at 6 months, while 1 site remained infected by all 3 species at 12 months. Of the 11 sites infected by all 3 species at baseline, 2 sites remained infected by 1 species and 1 site by 2 species at 1 month. No site was infected by all 3 species through 6 months post-treatment. By 12 months, 10 of the 11 sites originally infected by all 3 species became reinfected by at least 1 species (4 sites with 1 species, and 3 sites with 2 and 3 species, respectively).

**Effect of therapy on individual species.** Since the minimum sensitivity of the DNA probe assay was 6,000 cells/site, sites reported as negative following therapy (< 6,000 cells/site) were considered to have a value of 6,000 cells/site for computation and statistical purposes. Thus, the means illustrated for each species are likely larger than the true mean.

The mean number of Aa, Pg, and Pi for the 41 sites in 6 patients at each examination period is shown in Figure 1. The mean number of Aa was reduced from 26.5 ± 7.3 × 10^3 at baseline to 6.2 ± 0.1 × 10^3 at 1 month, 7.0 ± 0.8 × 10^3 at 3 months, 8.2 ± 1.7 × 10^3 at 6 months, and 9.8 ± 2.1 × 10^3 at 12 months. Since only 14 sites in the 6 subjects were infected with Aa at baseline, the distribution of Aa among subjects and among treatment groups was determined. As shown in Table 5, Aa was found in only 3 of the 6 patients at baseline, 1 of 6 at 1 week, 2 of 6 at 1 month, 2 of 6 at 3 months, 2 of 6 at 6 months, 1 of 6 at 9 months and 2 of 6 at 12 months. The individual patients infected by Aa at 1 week and 1 month were different than those infected at baseline, while both patients...
infected at 12 months were also infected at baseline. Furthermore, the infected sites were distributed across all treatment groups (data not shown).

Although 6/41 and 5/41 sites remained infected with Pg at 1 week and 1 month, respectively (Table 2), the magnitude of the infection was reduced from a total mean of 182.5 ± 30.5 × 10³ at baseline to 13.9 ± 4.4 × 10³ at 1 week and 7.6 ± 1.0 × 10³ at 1 month, rising to 49.3 ± 21.5 × 10³ at 9 months and 62.6 ± 25.7 × 10³ at 12 months (Fig. 1). Likewise, the total group mean Pi/site was reduced from a total mean of 74.7 ± 13.1 × 10³ at baseline to 7.6 ± 0.8 × 10³ at 1 month, 7.7 ± 0.9 × 10³ at 6 months and 11.5 ± 3.4 × 10³ at 12 months.

Effect of reinfection on clinical parameters. Since irrigation appeared not to affect the rate of reinfection, the 4 treatment groups were analyzed as a single group to determine the effect of reinfection on clinical parameters. When the number of reinfecting target species at 12 months was compared to preoperative plaque and gingival indices, no statistically significant relationship was observed (P = 0.56 and P = 0.67 for plaque and gingivitis, respectively).

However, when gingival fluid flow as measured in Periotron units at baseline was compared with the number of infecting species at 12 months, a highly statistically significant relationship was observed (Fig. 2A; P = 0.0009). The mean gingival fluid flow for uninfected sites was 147 ± 18, while the mean gingival fluid flow for sites with 1, 2, and 3 infecting species was 235 ± 56, 267 ± 50, and 612 ± 121, respectively.

Similarly, a statistically significant relationship was seen when mean probing depth at baseline was compared with the number of infecting species (P = 0.0009, Fig. 2B). The mean probing depths for uninfected sites were 3.6 ± 0.3 mm, while the mean probing depth for sites with 1, 2, and 3 infecting species was 5.0 ± 0.6, 5.7 ± 1.1, and 7.3 ± 1.1 mm, respectively.

In contrast to probing depth, no statistically significant relationship between mean attachment gain (baseline to 12 months) and number of infecting species (Fig. 2C, P = 0.31) was observed. The mean attachment gain for uninfected sites was 1.4 ± 0.3 mm, while the mean attachment gain for sites with 1, 2, and 3 infecting species was 0.4 ± 0.6, 0.3 ± 0.7, and 0.5 ± 0.3 mm, respectively. However, when mean attachment gain was related to uninfected versus reinfected sites without regard to the number of species present, a statistically significant relationship was observed (P = 0.05, Fig. 3). The 24 sites that had not become reinfected had a mean attachment gain of 1.38 ± 0.32 mm, while the 17 reinfected sites gained only 0.41 ± 0.35 mm.

Predictors of Reinfeciton
To determine if significant differences in clinical parameters at baseline might be prognostic of reinfection, the baseline clinical parameters of the 24 uninfected sites were compared with the baseline clinical measures of the 17 reinfected sites. As shown in Table 6, reinfected sites had a significantly greater baseline gingival fluid flow and probing depth, while uninfected sites had a significantly greater baseline gingival index.

Lastly, the number of infecting species at baseline was compared with the number of sites reinfected at 12 months by chi-square. Of the 9 sites infected by a single
DISCUSSION

Little information is available regarding the prevalence of microbial pathogens in patients previously treated for adult periodontitis,\textsuperscript{24,28–30} who do not subsequently comply with the maintenance recall regimen.\textsuperscript{31–38} Lack of compliance with the prescribed maintenance intervals has been associated with recurrence of the disease process and increased risk for tooth mortality.\textsuperscript{31,32} The recurrence of periodontitis in these non-compliant patients may be attributed to opportunistic overgrowth by pathogenic bacterial flora that have survived periodontal therapy or to reinfection of the treated sites by putative pathogens. In a previous report, we assessed the survival rate of Aa, Pg, and Pi following thorough scaling, root planing, and a single episode of intra-pocket irrigation with antimicrobial agents.\textsuperscript{25} Several sessions of root debridement led to reduction below detectable levels of the target organisms in 75% and 67% of the sites at 1 week and 1 month postoperatively, despite the irrigant used. However, the target species did survive in some sites, but at significantly reduced but detectable numbers.\textsuperscript{25} The current report measures the repopulation rate of 41 pockets in 6 of these patients by Aa, Pg, and Pi over 1 year in the absence of supportive periodontal care. Neither systemic antibiotics nor antimicrobial oral rinses were used during this period.

Lack of supportive maintenance therapy led to a tendency of regression toward baseline values for the mean plaque index, the mean gingival index, and the gingival fluid flow (Table 1). However, while some sites rebounded to pretreatment clinical parameters, this trend was not universal. The mean reduction in probing depth of 1 to 2 mm and the gain in clinical probing attachment of 1 mm observed one month postoperatively was generally maintained for most sites throughout the study (Table 1).

The effects of therapy on Aa, Pg, and Pi in the absence of supportive maintenance therapy were more transient than the clinical improvements observed.\textsuperscript{8,25,39,40} While therapy resulted in prolonged suppression of the tested species, they were detected at higher levels at the end of the observation period (Fig. 1). Whereas the level of the target bacterial species fluctuated greatly in some sites during the experimental period, it was not always accompanied by changes in clinical parameters.

Aa was detected in 14 sites at baseline, in 3 pockets at 1 week post-operatively, and in 7 sites (50%) at the end of the study (Table 2). However, the sites that harbored Aa postoperatively were not always the original sites nor in the same patient infected at baseline (Table 5). Patient 5 in this study tested negatively for Aa at baseline, but this species was detected in several postoperative samples obtained from this patient. The new presence of Aa in some previously uninfected sites could be attributed to exogenous new infection, repopulation of the pocket by Aa that was present within the gingival tissue at baseline, and thus beyond the reach of the sampling method, or the shortcoming of the therapeutic modalities utilized in this study.\textsuperscript{25} However, evidence is lacking to suggest that bacterial invasion persists following thorough scaling and root planing. Repopulation of the treated site may represent an opportunistic overgrowth of small colonies of Aa previously present in the pocket that were below the

Table 6. Clinical Predictors of Reinfection (comparison of baseline clinical parameters between uninfected and reinfected sites at 12 months)

<table>
<thead>
<tr>
<th>Reinfection at 12 Months</th>
<th>N</th>
<th>Plaque</th>
<th>Gingivitis</th>
<th>Gingival Fluid</th>
<th>Probing Depth</th>
<th>Attachment Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>17</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>459 ± 69*</td>
<td>6.8 ± 0.4*</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>1.4 ± 0.2</td>
<td>1.8 ± 0.1*</td>
<td>294 ± 46*</td>
<td>5.3 ± 0.3</td>
<td>4.4 ± 0.4</td>
</tr>
</tbody>
</table>

\*P < 0.05.
quantitative limit (6,000 cells) of the sensitivity of the DNA probe technology. Renvert and coworkers detected this species in 74% of the sites 6 months following root debridement by ultrasonic scaler and concluded that mechanical therapy, including subsequent surgery, is ineffective against this pathogen. Nieminen et al. have reported recently that non-surgical therapy alone is ineffective in eliminating Aa or Pg in a patient population with adult severe periodontitis. In contrast, a single episode of supragingival scaling combined with oral hygiene instructions provided a considerable antimicrobial effect in 10 subjects with a high prevalence of Pi, Pg, and Aa but with minimal periodontal destruction. Although Pg and Pi could still be detected in the majority of patients, the number of positive samples and bacterial counts were markedly lower at the end of 1 month observation period. In regard to Aa, the number of positive samples was reduced from 43% to 15%. Our data confirm previous reports of persistence of Aa following periodontal therapy.

Treatment was more effective in reducing Pg and Pi below detectable levels. Pg was detected in 33 sites at baseline and in only 12 pockets (36.4%) at 1 year postoperatively. Pi was the most prevalent species at baseline colonizing 37 sites, but was detected in only 12 pockets (32.4%) at the end of the study (Table 2). These data indicate differing response to scaling and root planing by different bacterial species.

The Predictors of Reinfection

One of the questions that this study attempted to answer was which baseline clinical and/or microbial parameter was prognostic of re-infection of the treated lesion. Identifying risk factors in treated populations may have important preventive and therapeutic implications.

Lack of maintenance therapy resulted in only 17 of the original 41 sites (41.5%) becoming reinfected by the tested pathogens at the end of the study (Table 6). Because a single episode of pocket irrigation with antimicrobial agents appeared not to affect the rate of re-infection, the 4 treatment modalities were grouped together and analyzed as a unit. While the plaque index and the clinical probing attachment loss at baseline were not statistically different at the 41 selected sites, the reinfected pockets exhibited deeper probing depths (mean 6.8 vs. 5.3 mm) and higher values for gingival fluid flow preoperatively (Table 6). These clinical parameters (probing depth and gingival fluid flow) could serve as predictors and/or risk factors of future re-population of the treated pocket by pathogenic bacteria in the absence of supportive therapy. Preoperative probing depth was also identified as a risk factor by Listgarten et al. in recurrence of periodontitis in a patient population who received periodic maintenance care. Attempts to set prognostic criteria for efficacy of non-surgical therapy in a patient population with advanced periodontitis have been reported recently by Nieminen et al. They identified the following variables as prognostic of failure of non-surgical therapy: simultaneous presence of multiple deep periodontal pockets (> 6 mm at 10% of the sites) coupled with subgingival colonies of Aa, alone or in clusters with Pg. Whether periodontal surgery aimed at pocket elimination or reduction could prevent or significantly postpone repopulation of the non-surgically treated pocket in a patient population with adult periodontitis is unknown and requires additional studies. The potential risk factor of furcation invasions for re-infection by these pathogens is also unknown. Although some test sites were multi-rooted teeth (13 multi-rooted teeth vs. 28 single rooted teeth), no attempts were made to obtain bacterial samples from the intraradicular areas of these teeth. Interestingly, the sites that remained free of the tested pathogens exhibited more gingivitis at baseline than reinfected sites (Table 6). The significance of the severity of existing gingivitis that accompanies adult periodontitis on the repopulation rate of the treated lesion by pathogenic bacteria requires additional studies. Data from the Michigan studies indicate lack of significance of recurrent gingivitis on treatment outcomes in a patient population enrolled in a longitudinal periodic supportive maintenance program.

Microbial Parameters

Pockets that were infected at baseline by Aa were more susceptible for re-infection than sites infected by either Pg or Pi (Tables 2 and 5). Another risk factor for re-infection relates to the combinations of these pathogens. Sites that harbored the cluster of 3 pathogens at baseline were more prone to re-infection at the end of the observation period versus sites that harbored only 1 or 2 species preoperatively (Table 4). However, in a treated patient population enrolled in a periodic maintenance program described by Listgarten et al., neither the presence nor the absence of Aa or Pi was found to be a sensitive or specific means of predicting disease recurrence. In regard to Pg as a risk factor, no data were available because of the low recovery of this species in this maintenance population. A recent report by Gunsolley et al. strongly implicated Pg, but not Aa, as a risk factor and a predictor of future attachment loss during the maintenance phase of therapy in a patient population with rapidly progressive periodontitis. Sites that were infected with this pathogen lost 0.5 to 4.5 mm of attachment during a 1-year observation period. Although some of these changes may be attributed to measurement error, these results clearly suggest that current maintenance therapy does not prevent disease recurrence or tooth loss in some patients.

The proportions of spirochetes in the subgingival plaque offered some promise as a marker of disease recurrence in patients not receiving periodic maintenance prophylaxis. However, their prognostic value was great-
ly diminished when the patients received regular supportive maintenance care. Clearly, the predictability of periodontal therapy would be greatly enhanced if our understanding of the mechanisms that promote survival of pathogenic bacterial species during periodontal therapy is increased and the process of repopulation and reinfection of the treated lesion is better understood. More research is needed to identify the potential value of various pathogenic bacterial species as markers, risk factors or indicators of recurring disease activity in different patient populations enrolled in longitudinal supportive maintenance programs.

However, it appears that the mere presence of pathogenic bacterial species in the subgingival milieu does not always indicate a disease process and that treatment success may not require total eradication of these microorganisms. Current data suggest that the presence of bacterial pathogens is necessary but not sufficient for disease activity to occur. Their presence in some pockets following successful therapy, albeit at significantly reduced numbers, may represent a "carrier state" or dormant stage, with the potential to exacerbate or to serve as a seeding source for infection in other sites in the same individual. The threshold level at which these species indicate pathology is unknown and requires additional studies. However, threshold values for a given pathogen may not be independent of other putative bacterial species, since certain bacterial combinations are more virulent than the individual species alone. Also, threshold values for a given pathogen are likely not to be independent of the host response.

The Effects of Reinfec tion on Treatment Outcome
Repopulation of the periodontal pocket by significant numbers of pathogenic bacteria following therapy has long been considered a risk factor for recurrence of the disease process. The process of repopulation of the subgingival environment by pathogenic bacteria following therapy is not well understood. Repopulation has been reported to occur in the presence of good plaque control or poor plaque control, following a single session or after several sessions of scaling and root planing, and in single-rooted and multi-rooted teeth. It may occur following periodontal-access surgery and regenerative procedures. Recolonization of the subgingival environment by putative pathogens may also occur following different regimens of systemic administration of antibiotics, controlled-release local delivery systems, or pocket irrigation. Recolonization may be attributed to the composition and virulence of the subgingival and intragingival microbial species. Resistance to therapeutic measures and survival of these pathogens in significant numbers, or repopulation of the treated sites by these bacteria, constitute a risk factor that may lead to deleterious effects on the outcome of periodontal therapy. The results of this study support these observations (Fig. 2B). The 24 sites that did not harbor these pathogens at the end of the study had a mean attachment gain of $1.38 \pm 0.32$ mm, while the 17 reinfected sites gained only $0.41 \pm 0.35$ mm (Fig. 3). This rebound towards baseline values points to the significance of periodic maintenance care in the treatment of periodontal diseases.

Conclusions
This study was conducted over a 1-year observation period on only 41 sites in 6 patients and therefore must be interpreted with caution. A larger sample size may have revealed smaller differences rather than the major effects reported here. However, even given the limitations of the small sample size, several conclusions can be reached:

1. A single episode of pocket irrigation with antimicrobial agents following thorough scaling and root planing did not affect the rate of repopulation of periodontal pockets by the tested pathogens.

2. Thorough scaling and root planing has a lasting suppressive effect on selected periodontal pathogens for most sites in patients with adult periodontitis. The reduction in the bacterial load and the concomitant reduction in inflammation and probing depth seen at 1 month were basically maintained at 1 year, with the majority of previously infected sites no longer yielding positive detection.

3. Preoperative probing depth, the amount of gingival fluid flow, and the composition of the subgingival microflora may serve as predictors for reinfection in the absence of maintenance care.

4. Reinfection of the treated sites by Aa, Pg, and/or Pi may constitute a risk factor that diminishes the effect of non-surgical therapy in the absence of maintenance care.

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