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Passive Immunization with Monoclonal Antibodies against *Porphyromonas gingivalis* in Patients with Periodontitis

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Selective inhibition of recolonization by *Porphyromonas gingivalis* was investigated by topical application of monoclonal antibody (MAb). To select a MAb to *P. gingivalis* with the potential for recognizing most strains of *P. gingivalis*, we examined seven MAbs, one of which (MAb 61BG 1.3) recognized all 22 laboratory strains and serotypes of *P. gingivalis* tested as well as 105 human clinical isolates. A comparative study of the number of *P. gingivalis* bacteria identified by conventional culture and immunofluorescence with MAb 61BG 1.3 showed a very significant correlation between the two methods (Spearman $r = 0.85$, $P < 0.001$). Fourteen patients with periodontitis, who harbored *P. gingivalis* in their subgingival plaque, were treated by root planing and with metronidazole to suppress any detectable *P. gingivalis*. In this double-blind study, the patients were then divided randomly into two groups; one was treated with MAb to *P. gingivalis*, and the other was treated with saline. Each patient had four subgingival applications of 3 μg of MAb (or saline) per tooth at 1, 3, 7, and 10 days after *P. gingivalis* was suppressed. The number of *P. gingivalis* bacteria was then monitored, and significantly less recolonization of the sites with the most severe periodontitis was found in the MAb-treated patients than in the control patients ($P < 0.01$). This was evident at 6 and 9 months after the application of MAb, but by 12 months, *P. gingivalis* was also found to recolonize these sites in two of the MAb-treated patients. The effect of MAb was specific to *P. gingivalis*, since the numbers of spirochetes were not significantly different between the two groups. However, no significant difference in any clinical periodontal indices between the immunized and control patients at 6 and 12 months was observed. This is the first demonstration that a putative periodontal pathogen can be selectively prevented from recolonization for up to 9 months in sites with the most severe periodontitis. This strategy could be used to establish directly in humans whether a microorganism is involved in the pathogenesis of periodontitis, by repeated application of the corresponding MAb at about 6-month intervals and by comparing the clinical indices between the MAb-treated and control patients.

Porphyromonas gingivalis is one of many subgingival microorganisms which has been associated with human periodontal disease and is thought to be a major etiological agent in human adult periodontitis (10, 25). Oral infection of nonhuman primates by *P. gingivalis* caused destructive disease in a ligature-induced model of periodontitis (11). Active immunization with formalinized intact *P. gingivalis* prior to infection of the nonhuman primates raised their serum and salivary antibody responses to the microorganism and reduced colonization of their subgingival plaque by *P. gingivalis* (5, 21). Of the two groups using this model, both found that immunization was associated with a reduction in colonization by *P. gingivalis*. However, one group found that this prevented the development of destructive periodontal disease (21), while the other group found no protection against periodontitis (5). Although the model is the best available, macaques are normally not susceptible to periodontitis, and colonization with *P. gingivalis* is fraught with difficulties and requires application of ligatures for plaque accumulation. We have therefore searched for an alternative way of studying the role of *P. gingivalis* in periodontal disease. From the limited options available, we decided to use the passive immunotherapy strategy developed for prevention of colonization by *Streptococcus mutans* in primates (14). Topical application of monoclonal antibodies (MAbs) specific to *S. mutans* (13) or bovine milk antibodies to *S. mutans* (19) prevented colonization by that microorganism and the development of caries in nonhuman primates (13) or gnotobiotic rats (19). Prevention of colonization or recolonization by *S. mutans* was then demonstrated in a series of experiments with humans (17, 18). The most effective results were achieved by suppressing the microorganism to undetectable levels before applying the MAbs.

With the success of active immunization against *P. gingivalis* and passive immunization against *S. mutans* demonstrated in nonhuman and human primates, we explored the possibility of preventing recolonization by *P. gingivalis* in humans and assessing its effect on periodontal disease by passive immunization with MAbs to *P. gingivalis*. We applied specific MAbs subgingivally in patients with periodontitis who had *P. gingivalis* in their subgingival flora.

MATERIALS AND METHODS

Selection of MAb for passive immunization. Selection of a MAb suitable for passive immunization is crucial, and there appear to be two essential requirements, namely, (i) that the MAb recognizes most if not all strains of *P. gingivalis* found in human periodontal disease and (ii) that the MAb prevents adhesion of *P. gingivalis*. Seven MAbs, including MAb 61BG 1.3 and MAb 64BG (7) as well as five MAbs raised at Guy's Hospital (13a), were screened by immunofluorescence (IF) with strains of *P. gingivalis*, and MAb 61BG 1.3 was selected as that best fulfilling the first requirement mentioned above. The second requirement was not tested directly, and we relied on the putative high avidity of the selected immunoglobulin G1 (IgG1) MAb 61BG 1.3 from its high titer of $1{:}10^5$ (2). Furthermore, MAb 61BG 1.3 recognizes a hemagglutinating epitope at the surface of *P. gingivalis* (2), which may function as one of the virulence factors in colonization of this organism.

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Microbial specificity testing of MAb 61BG 1.3 by IF. Twenty-two laboratory strains or serotypes of *P. gingivalis* were obtained from various sources, and 105 human *P. gingivalis* isolates and three monkey strains were isolated at United Medical and Dental Schools of Guy's and St. Thomas' Hospitals. They were grown on enriched blood agar (1) containing kanamycin for 4 days at 37°C in an atmosphere of 80% N_2, 10% CO_2, and 10% H_2. All cells were washed three times in phosphate-buffered saline (PBS) and then fixed to the wells of Teflon-coated microscope slides (C. A. Hendley, Loughton, United Kingdom) at 56°C for 30 min. The specificity of MAb 61BG 1.3 was investigated by a modification of the three-stage IF technique (6, 7). Briefly, undiluted culture supernatant containing MAb 61BG 1.3 or the irrelevant IgG1 MAb to the human papilloma virus was incubated on slides for 30 min at 37°C. Preliminary titrations of the MAb supernatant had established that optimum results were obtained with undiluted reagents. After two 10-min washes in PBS, biotin-conjugated goat anti-mouse IgG (Sigma Ltd., Poole, United Kingdom) diluted 1:200 in borate-buffered saline with 1% skim milk powder and 0.05% Tween 20 was incubated on slides at 37°C for 30 min. After two further washes in PBS, the slides were incubated with avidin-labelled fluorescein isothiocyanate at a dilution of 1:300 for 30 min. The slides were then washed three times in PBS for 15 min before the application of a drop of 1:1 (vol/vol) PBS-glycerol containing a reagent preventing fading (Citifluor, Canterbury, United Kingdom). The slides were mounted with a glass coverslip before viewing under an oil-immersion objective (×1,000) with a Jenamed Zeiss fluorescent microscope. MAb 61BG 1.3 was considered to recognize *P. gingivalis* if it demonstrated a clear ring-like staining pattern by IF.

Comparison of IF and anaerobic culture for the detection of *P. gingivalis* in subgingival plaque. (i) Patients and controls. We examined plaque collected from patients with a range of periodontal conditions which were weighted towards samples from patients considered to be suitable for passive immunization. Sixteen adults aged from 22 to 80 years were selected from dental personnel and from patients referred to United Medical and Dental Schools of Guy's and St. Thomas' Hospitals for specialist treatment. Of these, four dental staff members aged 22 to 64 years had mild gingivitis or clinically healthy gingivae, nine patients aged 33 to 80 years had periodontitis which ranged in severity from early to moderate loss of attachment, and three patients aged 35 to 44 years had advanced periodontitis.

(ii) Collection and processing of plaque samples. Subgingival plaque samples were collected on three sterile paper points from the most severely affected interproximal site on a first permanent molar and placed in 1 ml of reduced transport fluid (15). The samples were transferred immediately to an anaerobic environment, where they were vortex mixed and homogenized. Part of each sample was serially diluted and plated on enriched blood agar and also on selective kanamycin blood agar (1). The plates were incubated at 37°C for 10 days, after which the number of anaerobic CFU was counted on the nonselective plates and pigmented colony types were described and counted on the selective media. One in five of each pigmented colony type was subcultured until pure growth was obtained. The identity of isolates was confirmed as *P. gingivalis* only if they were gram-negative coccobacilli producing trypsin-like activity but which did not produce catalase or ferment a range of sugars. Doubling serial dilutions of the plaque samples were also fixed to the wells of Teflon-coated microscope slides and processed for IF as described above.

(iii) Microscopy. Slides were coded and read blind by one of us (V.B.). When a Reichert dark-field microscope was used, a dilution was chosen for each slide to enable the microorganisms to be readily counted. Only bacterial shapes with bright halos and dark centers or with the morphology of spirochetes were counted. Five fields with approximately 100 microorganisms per field were counted, and the mean number of microorganisms per field was calculated. Ten fields were observed at the same magnification with a Jenamed Zeiss fluorescence microscope, and the mean number of *P. gingivalis* bacteria per field was calculated.

Passive immunization with MAb 61BG 1.3. (i) Selection of patients for passive immunization. We enrolled 14 patients, aged 25 to 55 years and referred for periodontal treatment to Guy's Dental Hospital, in whom *P. gingivalis* was detectable and constituted at least 1% of the microbial flora in plaque samples by the IF assay. Patients had at least 20 standing teeth and had already undergone initial periodontal therapy involving oral hygiene instruction and scaling but still had at least two probing pockets with a depth of 5 mm or more which bled after probing. Patients with loss of periodontal attachment extending between the roots of multirooted teeth (grade iii furcation involvement) were excluded from the investigation. Ethical committee approval was obtained before starting this study.

(ii) Clinical evaluation. We assessed the clinical condition of the patients before treatment and 1.5, 6, and 12 months after passive immunotherapy. All clinical parameters were recorded for the four interproximal sites and the midbuccal and midlingual sites of all standing teeth. Acrylic reference stents were constructed for all patients, and measurements were made at marked sites from the stent to the gingival margin and from the stent to the base of the pocket with a controlled-force probe (model 250; Vine Valley Research, Middlesex, N.Y.) set at a force of 25 g with a probe tip diameter of 0.45 mm. Measurements were rounded down to the nearest millimeter. To indicate the level of inflammation, bleeding after probing was assessed as profuse, discrete (pinpoint), or absent (23). The presence or absence of supragingival plaque was also recorded.

(iii) Assessment of *P. gingivalis* and spirochetes. We assessed the number of *P. gingivalis* bacteria and spirochetes in subgingival plaque samples collected from sites with periodontal disease which had probing depths of ≥4 mm and bleeding after probing or from clinically healthy sites. This was carried out before treatment, before the first immunization, and then at 0.5, 1.5, 3, 6, 9, and 12 months after the completion of immunization. Supragingival plaque was removed from the sampling sites with a curette. Samples were collected on three sterile paper points inserted into the pocket or crevice for 10 s. The number of *P. gingivalis* bacteria was assessed by IF with MAb 61BG 1.3 as described above. The total number of microorganisms and the number of spirochetes indicated by their morphology under dark-field microscopy were also determined. Spirochetes were enumerated since they are associated with clinical conditions similar to those of *P. gingivalis* and are also susceptible to mechanical periodontal therapy and metronidazole treatment (9, 16). It was considered that this might indicate whether any prevention of recolonization of *P. gingivalis* by the MAb was specific to this organism or if the MAb might affect other microorganisms, such as spirochetes, by changing the pocket environment.

(iv) Preparation of MAbs. MAb 61BG 1.3 was purified from culture supernatant fluid by precipitation with a saturated solution of ammonium sulfate and ion-exchange chromatography on a DEAE-cellulose column (Whatman, Maidstone, United Kingdom) in 0.01 M Tris-phosphate (pH 7.0). Partially purified MAb was then treated with 0.3% tri(*n*-butyl)-phosphate and 0.2% sodium cholate to ensure the removal of any lipid-enveloped viruses and passed through a Sephadex G-25 column with 0.02 M Tris-HCl (pH 7.2)–0.02 M sodium citrate–0.1 M sodium chloride (12, 22). Purified MAb was tested for and shown to be free from infectious agents by Q1 Biotech Ltd. (Glasgow, Scotland) according to the guidelines on the production and quality control of murine MAbs intended for use in humans issued by the Commission of the European Communities Committee for Proprietary Medicinal products (4). The MAb solution was made up in sterile saline to contain 100 μg of the MAb per ml.

(v) Treatment to suppress *P. gingivalis*. We first reduced oral colonization by *P. gingivalis* to the lowest possible level by routine periodontal treatment involving oral hygiene advice and thorough root planing, followed by a 7-day course of metronidazole (200 mg three times a day) to target any microorganisms colonizing soft tissues (24). We monitored all patients at 0.5, 1.5, 3, 6, 9, and 12 months after the first application of MAb, and any subsequent periodontal therapy was restricted to the supragingival region, leaving the subgingival flora to recolonize undisturbed. Previous work had indicated that recolonization by *P. gingivalis* following scaling and metronidazole treatment could be expected within weeks (16), so the aim of treatment with MAb 61BG 1.3 was to explore whether recolonization with *P. gingivalis* could be prevented for a longer period.

(vi) Application of the MAbs. The patients were assigned on a double-blind basis to either the experimental or the control group. Patients in the experimental group were given 5 μl of MAb solution subgingivally at six sites around every tooth or a total of 30 μl (3 μg of MAb) per tooth. The control patients had saline applied in an identical manner, and all immunizations and sham immunizations were performed 1, 3, 7, and 10 days after the course of metronidazole treatment was completed. In view of concerns about the limited penetration of subgingival irrigants and the possibility of trauma, the solutions were applied subgingivally with a 0.5-mm-diameter blind-ended needle with a side port (Hawe-Neos Dental, Gentilino, Switzerland) specifically devised for irrigation of periodontal pockets and root canals. The solutions were dispensed from a 250-μl Hamilton (Reno, Nev.) syringe with a Hamilton repeating attachment. No patients reported any side effects after the immunizations.

(vii) Radioimmunoassay. To determine whether subgingival topical application of MAb 61BG 1.3 induced serum antibodies against the MAb, we collected venous blood before treatment and 1.5 and 6 months after the first topical application. The levels of serum IgG, IgM, or IgA antibodies to the murine MAb 61BG 1.3 were assessed by a radioimmunoassay. Aliquots of 0.1 μg of MAb 61BG 1.3 per ml were bound to the wells of microtiter plates which had been pretreated with poly-L-lysine. Sera collected before and after application of MAb 61BG 1.3 (or saline) were serially diluted from 1:10 to $1:10^6$ in PBS with 1% bovine serum albumin (with 0.05% Tween 20) and incubated on the plates for 1 h. After four washes with PBS and Tween 20, bound human antibody was detected by biotin-labelled goat anti-human IgG, IgM, and IgA (Sigma Ltd.). The anti-IgG and anti-IgA antibodies were used at a previously determined optimum dilution of 1:5,000, and the anti-IgM serum was used at a dilution of 1:7,500. After further washes, the plates were incubated for 1 h with 200 μl of ^{125}I-streptavidin (Amersham International Plc, Aylesbury, United Kingdom), which was adjusted to a mean level of activity of approximately 40,000 cpm. The plates were washed and left to dry overnight at room temperature, and the wells were counted in a gamma counter. Binding of the ^{125}I was expressed as counts per well and as a percentage of the ^{125}I activity applied to the wells. We examined the endpoints of the titration curves for sera from individual patients before and after experimental or control application. We also compared the levels of serum antibodies in experimental and control patients throughout the study.

Statistical methods. In the comparison of the levels of detection of *P. gingivalis* by IF and culture, the number and the proportions of samples identified as *P. gingivalis* were examined by the Spearman correlation test. The levels of *P. gingivalis* bacteria and spirochetes in immunized and sham-immunized subjects were analyzed by the Mann-Whitney U test at separate time intervals. The mean bleeding scores and plaque scores per patient, the mean numbers of pockets

TABLE 1. Comparison of anaerobic culture and IF with MAb 61BG 1.3 to detect *P. gingivalis* in plaque samples from 16 subjects[a]

Clinical condition	Anaerobic culture			Microscopy		
	Total anaerobes[b]	No. of *P. gingivalis*[b]	% *P. gingivalis*	Total dark-field count[c]	IF *P. gingivalis* count[c]	% *P. gingivalis*
Healthy gingiva or gingivitis	2.8	0.0	0.0	568.0	0.0	0.0
	0.2	0.0	0.0	518.4	0.0	0.0
	36.8	0.0	0.0	700.8	0.0	0.0
	70.0	0.0	0.0	890.4	0.0	0.0
Early to moderate periodontitis	680.0	111.3	16.4	1,267.2	153.6	12.1
	122.5	18.5	15.1	1,817.6	102.4	5.6
	2.7	0.0	0.0	164.0	0.6	0.4
	60.0	0.0	0.0	444.0	0.4	0.1
	0.7	0.0	0.0	229.6	0.2	0.1
	327.5	101.7	31.0	170.4	52.0	30.5
	0.2	0.0	0.0	75.6	0.7	0.9
	13.4	0.0	0.0	146.0	0.4	0.3
	575.0	0.0	0.0	1,744.0	1.6	0.1
Advanced periodontitis	197.5	27.8	14.1	3,852.8	112.0	3.3
	890.0	400.4	45.0	3,814.4	364.8	9.6
	59.8	22.1	37.0	942.4	40.0	4.2

[a] The Spearman correlation coefficients were as follows: (i) between total anaerobic CFU and total dark-field counts, 0.70 ($P < 0.01$); (ii) between *P. gingivalis* CFU and IF counts, 0.86 ($P < 0.001$); and (iii) between % of *P. gingivalis* in samples by culture and that by IF, 0.85 ($P < 0.001$).
[b] CFU (10^5) per sample.
[c] Mean number of cells per microscope field × dilution factor.

deeper than 3 mm, the mean number of sites with changes in attachment level of 2 mm or more, the probing depths at the single diseased sampling sites, and the levels of anti-MAb 61BG 1.3 antibodies at a serum dilution of 1:1,000 were tested by repeated-measure two-way analysis of variance (ANOVA) to establish whether there were any significant differences in these parameters between the immunized and sham-immunized patients or between any time points in the study. The number of teeth per patient was used as a covariate in the two-way ANOVA to account for differences between the two groups in the number of sites contributing to the scores. The distribution of bleeding and plaque at the single diseased sampling site was examined by chi-square tests for immunized and sham-immunized patients. The results of all tests were considered to be statistically significant when P was <0.05.

RESULTS

Specificity of MAb 61BG 1.3 for *P. gingivalis*. A ring-like staining pattern was found with MAb 61BG 1.3 applied to all isolates of *P. gingivalis*, which included 22 laboratory strains, 105 clinical isolates, and 3 monkey strains. However, the intensity of fluorescence varied between different strains of *P. gingivalis*. The results indicate that the determinant recognized by MAb 61BG 1.3 was expressed by all strains of *P. gingivalis* tested.

Selection of MAb 61BG 1.3 for passive immunization. MAb 61BG 1.3 was selected from seven MAbs after it recognized all strains and isolates of *P. gingivalis* with which it was tested and after it gave a bright ring-like fluorescence.

Detection of *P. gingivalis* in subgingival plaque. *P. gingivalis* was detected in 6 of the 16 subjects by both IF and culture, and the proportion of *P. gingivalis* in these 6 patients constituted over 1% of the total microbial flora by either method (Table 1). Three of those subjects had advanced periodontitis, and three had either moderate or early periodontitis. In six other subjects with either early or moderate periodontitis, *P. gingivalis* was detectable by IF but not by culture, and in those subjects, *P. gingivalis* constituted less than 1% of the sample (Table 1). Indeed, all patients with periodontitis revealed evidence of *P. gingivalis* by IF in their plaque, although the number of organisms was low in six patients. Only in the four subjects with healthy gingivae or mild gingivitis was *P. gingivalis* undetectable by either method. A very significant correlation was found between the number or proportion of *P. gingivalis* bacteria detected by culture and that by IF; the Spearman correlation coefficients were 0.86 and 0.85, respectively ($P < 0.001$). There was also a significant ranked correlation of 0.70 ($P < 0.01$) between the total anaerobic growth and the numbers of microorganisms detected by dark-field microscopy in each sample.

Recolonization. *P. gingivalis* was effectively suppressed by root planing and metronidazole treatment since the microorganism was not detectable by IF in samples collected from the most severely diseased site on a first permanent molar. Recolonization of this site by *P. gingivalis* was detectable in five of the seven controls at 6 and 9 months, when none of the immunized patients had detectable *P. gingivalis* (Fig. 1). The number of *P. gingivalis* in the five control patients increased at 6 and 9 months, but the organism was not detected in any of the immunized patients; this difference was significant (Mann-Whitney U test, $P < 0.01$). The highest numbers of *P. gingivalis* bacteria were recovered from one of these sites, which was suppurating, and this control patient was removed from the

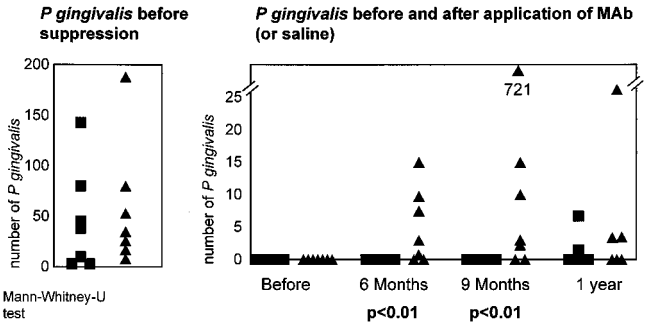

FIG. 1. Numbers of *P. gingivalis* bacteria in a sample collected from the most diseased site on a first permanent molar in 14 patients before treatment, after suppression of *P. gingivalis*, and at 6, 9, and 12 months after the first topical application of MAb 61BG 1.3 (■) or saline (▲). Mann-Whitney U tests showed significantly lower numbers of *P. gingivalis* bacteria in these samples 6 and 9 months after application of MAb 61BG 1.3 than after a saline placebo but no significant difference after 1 year.

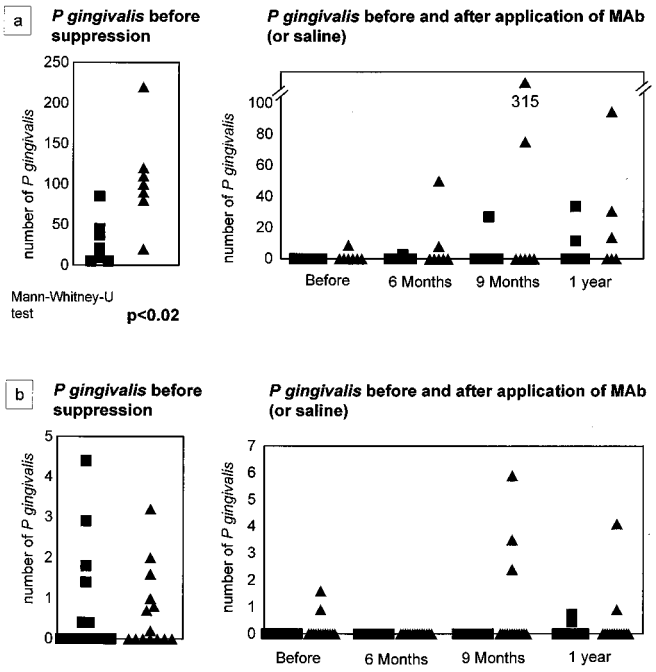

FIG. 2. Numbers of *P. gingivalis* bacteria in a sample pooled from three diseased sites (a) and two samples from healthy sites (b) in 14 patients before treatment, after suppression of *P. gingivalis*, and at 6, 9, and 12 months after the first topical application of MAb 61BG 1.3 (■) or saline (▲). Before treatment, Mann-Whitney U tests showed significantly higher numbers of *P. gingivalis* in samples pooled from diseased sites but not from healthy sites in patients subsequently sham immunized with saline. However, there were no significant differences between the groups in any of the samples after suppression of *P. gingivalis* or after topical applications.

rest of the study for further treatment. One year after immunization, two of the seven patients immunized with MAb 61BG 1.3 had also started to recolonize, and the effect of the MAb 61BG 1.3 application was no longer significant (Fig. 1). The numbers of spirochetes in the same samples were never significantly different at any stage of the study in the immunized and sham-immunized patients.

Fewer of the samples pooled from three diseased sites or collected from clinically healthy sites were recolonized with *P. gingivalis* than those collected from the most severely diseased site on a first permanent molar. In samples pooled from three diseased sites (on a molar, premolar, and incisor or canine), we found significantly higher numbers of *P. gingivalis* bacteria before treatment ($P < 0.02$) in patients later assigned blindly to the control group (Fig. 2a). However, after treatment to suppress *P. gingivalis*, in all but one control patient, this organism was not detectable (Fig. 2a). Recolonization of these samples occurred in fewer patients than in the samples collected from the worst site on a first permanent molar. *P. gingivalis* was detected in two sham-immunized patients and one immunized patient at 6 and 9 months and in three sham-immunized and two immunized patients at 1 year (Fig. 2a). Samples collected from a single healthy site or pooled from three healthy sites (Fig. 2b) had no detectable *P. gingivalis* 6 months after immunization. However, 9 months after immunization, *P. gingivalis* was detected in three of these samples from sham-immunized patients but in none of the samples from immunized patients. One year after immunization, two patients in each group showed low levels of recolonization by *P. gingivalis* in samples from healthy sites (Fig. 2b).

Clinical evaluation. Significant improvement following

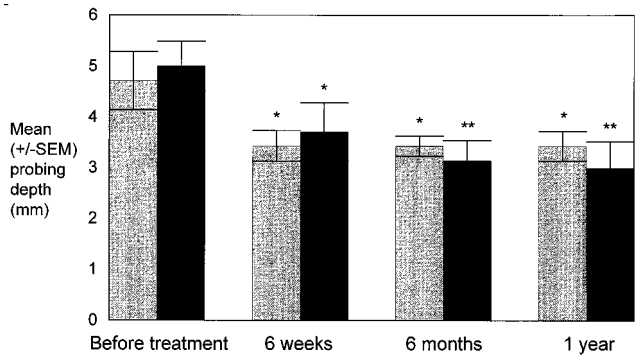

FIG. 3. Mean probing depth (± standard error of the mean) at the diseased sampling site on a first permanent molar before treatment and at intervals after application of MAb 61BG 1.3 (▨) or saline (■). Two-way ANOVA showed no significant differences in the probing depth at these sites between immunized and sham-immunized patients at any time in the study. However, within both groups, there were significant reductions in the probing depth after treatment (**, $P < 0.01$; *, $P < 0.05$).

treatment was found in the general periodontal condition of immunized and sham-immunized patients as indicated by the numbers of deep pockets ($P < 0.001$) and sites with inflammation ($P < 0.001$ to 0.05) by applying repeated-measures two-way ANOVA. However, there were no significant differences between immunized and sham-immunized patients in the numbers of pockets deeper than 3 mm, in the level of inflammation indicated by the bleeding scores, or in the numbers of sites with changes in probing attachment level greater than 2 mm at any time after immunization. The lack of significant differences observed after immunization was confirmed by ANOVA with either pretreatment levels of the clinical variables as covariates or with the level of interdependent clinical variables (e.g., probing depth and inflammation) as covariates.

There were also no significant differences between immunized and sham-immunized patients in the probing depths at the single diseased sampling sites (Fig. 3) where significant differences in recolonization by *P. gingivalis* were detected 6 and 9 months after immunization. However, in both groups, significant reductions in the probing depths ($P < 0.01$ to 0.05) were observed after treatment (Fig. 3). There were also no differences in the distribution of inflammation at the sampled sites, indicated by the frequency of bleeding or profuse bleeding after probing immunized or sham-immunized sites (Table 2; 6-month data). Significant differences in the distribution of plaque were also not observed at these sites (Table 2).

TABLE 2. Frequency distribution of bleeding, profuse bleeding, and plaque at the single diseased sampling site 6 months after immunization

Condition	No. of subjects		Frequency by chi-square test[a]
	Immunized with MAb 61BG 1.3	Sham immunized	
No bleeding after probing	3	5	1.17 (P = ns)
Bleeding after probing	4	2	
No profuse bleeding after probing	6	7	1.08 (P = ns)
Profuse bleeding after probing	1	0	
Plaque absent	5	4	0.31 (P = ns)
Plaque present	2	3	

[a] ns, not significant.

Radioimmunoassays. No significant differences were found in the IgG, IgM, and IgA serum antibody titers to MAb 61BG 1.3 between the immunized and sham-immunized patients throughout the study, as analyzed by repeated-measure two-way ANOVA. In addition, the antibodies in each individual patient showed no increase in titer against MAb 61BG 1.3. These results suggest that four applications of MAb did not elicit antibodies to the murine MAb.

DISCUSSION

MAb 61BG 1.3 recognized all of the representative serotypes, strains, and 108 primate isolates of *P. gingivalis* tested, suggesting that this MAb recognizes a common and accessible B-cell determinant of *P. gingivalis*. The recognition of a common species-specific determinant by MAb 61BG 1.3 combined with its good cell surface staining properties and its recognition of a hemagglutinating protein (2) satisfied the criteria for choosing this MAb for passive immunization. The recognition of all tested strains of *P. gingivalis* in IF assays is consistent with a very significant correlation between the level of *P. gingivalis* detected in plaque by IF and that detected by a conventional culture technique. However, as in previous studies which compared IF using MAbs with culture techniques to identify *P. gingivalis* (6, 26), we also found that the organism was detected in more samples by IF than by culture.

Recolonization by *P. gingivalis* of the deepest pocket on a first permanent molar did not occur for 9 months after topical application of MAb 61BG 1.3, in comparison with recolonization after sham immunization ($P < 0.01$). This appeared to be specific, since no effect on the number of spirochetes was observed for the same samples. Recolonization occurred in fewer samples pooled from three deep pockets or collected from healthy sites than in those collected from the most severely involved sites, and the trend towards more rapid recolonization of the sham-immunized patients did not reach significant levels. There were no side effects reported by the patients or observed by the clinician, and increasing antibodies to the murine MAb were not detected in any of the patients after four subgingival applications of the MAb.

MAb 61BG 1.3 recognizes a hemagglutinating protease extract of *P. gingivalis* and can inhibit hemagglutination caused by the extract or by intact *P. gingivalis* (2). Hemagglutinins mediate adhesion which in *P. gingivalis* not only allows adherence to erythrocytes but also delivers the microorganism to a source of essential nutrients (3). The results of this study suggest that the biologically important hemagglutinin or protease of *P. gingivalis* can be blocked in vivo by means of MAb 61BG 1.3, and this prevents recolonization of the microorganism in subgingival plaque. This is consistent with the results with hamsters in which topical application of rabbit antiserum against the *P. gingivalis* hemagglutinin reduced colonization of teeth by exogenous *P. gingivalis* over 3 weeks (20). It is noteworthy that unlike MAb to the cell surface adhesin of *S. mutans* which prevented recolonization of this microorganism for up to 2 years (18), MAb to *P. gingivalis* was effective only for up to 9 months. However, these two investigations may have established a principle that both supragingival (*S. mutans*) and subgingival (*P. gingivalis*) plaque microorganisms can be prevented from recolonization by passive immunotherapy. The duration of prevention of recolonization by the two MAbs differed, and this may be related to a number of factors. The most significant might be the differences between the microbiota and environment of subgingival plaque and that of supragingival plaque, both in the type of microorganisms and in the immunological influence of predominantly crevicular fluid subgingivally and saliva with crevicular fluid supragingivally. It should also be borne in mind that *S. mutans* is a gram-positive organism, whereas *P. gingivalis* is a gram-negative organism, and that the mechanisms of action of the MAbs are different, as noted above.

No significant differences in any of the clinical indices were observed between immunized and sham-immunized patients at 6 and 12 months. However, the significant improvement in the periodontal health of patients in both groups was maintained for 1 year after routine treatment. Since rapid and detectable progression of periodontitis in untreated disease is relatively rare (8), it is well recognized that clinical changes, particularly in treated disease, may require longer periods of time to become evident. Active immunization of nonhuman primates which significantly reduced colonization by *P. gingivalis* was protective against progression of periodontitis in one study (21) but not in another (5). There are at least two possible interpretations of our results, as follows: (i) *P. gingivalis* alone plays no or a minimal part in the etiology of periodontitis and (ii) *P. gingivalis* is pathogenic if present in subgingival plaque in higher numbers than had recolonized in this study or for a longer period of time (over 1 year). A change in the virulence of the recolonized *P. gingivalis* will also need to be considered.

Selective inhibition of microbial recolonization by topical application of MAb might prove to be an effective means of examining each of the many microorganisms implicated in the pathogenesis of periodontitis. Prevention of recolonization of *P. gingivalis* or other periodontopathic microorganisms by application of MAb at about 6-month intervals for 2 to 3 years might establish whether that organism plays a part in the progression of periodontitis.

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