Nucleotide Sequence of the Porphyromonas gingivalis W83 recA Homolog and Construction of a recA-Deficient Mutant

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Degenerate oligonucleotide primers were used in PCR to amplify a region of the recA homolog from Porphyromonas gingivalis W83. The resulting PCR fragment was used as a probe to identify a recombinant λ DASH phage (L10) carrying the P. gingivalis recA homolog. The recA homolog was localized to a 2.1-kb BamHI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical to) the RecA protein from Bacteroides fragilis. No SOS box, characteristic of LexA-regulated promoters, was found in the 5′ upstream region of the P. gingivalis recA homolog. In both methyl methanesulfonate and UV survival experiments the recA homolog from P. gingivalis complemented the recA mutation of Escherichia coli HB101. The cloned P. gingivalis recA gene was insertionally inactivated with the ermF ermAM antibiotic resistance cassette to create a recA-deficient mutant (FLL33) by allelic exchange. The recA-deficient mutant was significantly more sensitive to UV irradiation than the wild-type strain, W83. W83 and FLL33 showed the same level of virulence in in vivo experiments using a mouse model. These results suggest that the recA gene in P. gingivalis W83 plays the expected role of repairing DNA damage caused by UV irradiation. However, inactivation of this gene did not alter the virulence of P. gingivalis in the mouse model.

Porphyromonas gingivalis, a black-pigmented, gram-negative anaerobe, has been implicated as an important etiological agent in adult periodontitis. This organism possesses several putative virulence factors (e.g., hyaluronidase, fimbiae, hemagglutinin, capsule, and lipopolysaccharide) that can directly affect the periodontium or elicit host functions that result in destruction typical of advanced periodontitis (12, 23, 35). In addition, colonization of the inflammatory microenvironment of the periodontal pocket by this organism requires an ability to overcome oxidative stress resulting from bacterial metabolites generated from neutrophils (1) and occasional exposure to air (2). Reactive oxygen metabolites such as superoxide (O2⋅−), hydrogen peroxide (H2O2), and hydroxyl radical (-OH) are major components of the bactericidal activity of polymorphonuclear leukocytes (5, 32). The toxic oxygen metabolites can be neutralized by superoxide dismutase, catalase, and peroxidase, all of which are generally expressed by aerobic and many anaerobic bacteria (3, 31). Although P. gingivalis is oxygen tolerant (2) and expresses superoxide dismutase activity (6, 25, 26), it is missing catalase and peroxidase activity (2).

DNA damage is one of the lethal effects of oxygen metabolites. The recA gene product is a key protein in repair of DNA. In addition to being involved in homologous recombination, it is a regulatory protein that is induced by DNA damage and mediates, in Escherichia coli, the expression of more than 20 genes in the SOS regulatory network (reviewed in reference 24). Effects of SOS induction are many and include increased DNA repair, prophage induction, and/or an increased rate of mutagenesis (reviewed in reference 39). Since an important source of spontaneous DNA damage is attack by reactive oxygen species (15), it is reasonable to assume that DNA repair ability may play a role in the virulence of P. gingivalis. DNA repair ability may also be important for survival of P. gingivalis in the periodontal pocket, where a high level of inflammation and neutrophil infiltration occurs (19).

Here we report the nucleotide sequence determination of a recA homolog from P. gingivalis W83. This gene complemented the recA mutation of E. coli HB101. Using the cloned recA homolog, a recA-defective mutant was constructed by allelic exchange. This strain demonstrated an increased sensitivity to UV irradiation. Further, we found that the recA mutation did not affect the virulence of that mutant in a mouse model. This recA-defective strain of P. gingivalis W83 will facilitate genetic complementation analysis and vaccine development.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Porphyromonas gingivalis W83 was grown in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) supplemented with hemin (5 μg/ml), vitamin K (0.5 μg/ml), and cysteine (1%). E. coli strains were grown in Luria-Bertani (LB) broth (33). Unless otherwise stated, all cultures were incubated at 37°C. P. gingivalis was maintained in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Mich.) in 10% H2, 10% CO2, and 80% N2. Growth rates for P. gingivalis strains grown in BHI broth supplemented with vitamin K and hemin were determined spectrophotometrically (optical density at 660 nm).

DNA isolation and analysis. Porphyromonas gingivalis chromosomal DNA was prepared by the method of Marmur (22). Plasmid DNA extraction followed the alkaline lysis procedure of Birnboim and Doly (4). Plasmids were purified by cesium chloride-ethidium bromide centrifugation as described by Sambrook et al. (33). A λ DNA was prepared as described by Sambrook et al. (33). DNA was digested with restriction enzymes as specified by the manufacturer (GIBCO BRL, Gaithersburg, Md.). Southern blot transfer was done according to the method of Smith and Summers (37). DNA hybridization was done at 42°C with 50% formamide to allow a stringency of 80%. The blot was washed with 0.1% SSPE (×1 SSPE is 0.18 M NaCl, 10 mM NaPO4, and 1 mM EDTA [pH 7.7]) and 0.1% sodium dodecyl sulfate. DNA labeling and autoradiography were done as reported previously (21).

DNA sequencing. DNA from the 2.1-kb BamHI fragment containing the recA homolog from L10 was subcloned into pHCM19. Nucleotide sequences were determined by the dideoxy chain termination method (34) with a Perkin-Elmer (Foster City, Calif.) DNA sequencing kit using M13 forward (−40) and M13 reverse. The sequence was determined for both strands. A number of the clones sequenced did not correspond to the cloned sequence. This strain demonstrated an increased sensitivity to UV irradiation. Further, we found that the recA mutation did not affect the virulence of that mutant in a mouse model. This recA-defective strain of P. gingivalis W83 will facilitate genetic complementation analysis and vaccine development.
reverse (48 primers (United States Biochemical Corporation, Cleveland, Ohio) and analyzed on an Applied Biosystems (Foster City, Calif.) model 373A DNA sequencing system at the DNA core facilities of Virginia Commonwealth University (Richmond, Va.) and Loma Linda University (Loma Linda, Calif.). Oligonucleotide primers were synthesized at the DNA core facilities of Virginia Commonwealth University and Loma Linda University. Nucleotide sequences were analyzed with the Genetics Computer Group sequence analysis software package (8) and the MacVector sequence analysis software package for the Macintosh (International Biotechnologies Inc., New Haven, Conn.).

**PCR conditions.** Degenerate oligonucleotide primers were designed as previously described (9). PCR amplification was performed with a Perkin-Elmer Cetus DNA thermal cycler (Perkin-Elmer Corporation, Norwalk, Conn.) as described by Sambrook et al. (33). The reaction mixture (100 μl) contained 0.5 μg of each primer, 70 ng of template DNA, and 1 μl of Vent Polymerase (New England Biolabs, Beverly, Mass.). PCR consisted of 25 cycles with a temperature profile of 1 min at 94°C, 2 min at 48°C, and 3 min at 72°C. Total DNA from *P. gingivalis* W83 was used as a template. The PCR-amplified DNA product was purified by phenol-chloroform extraction and ethanol precipitation and was identified by agarose gel electrophoresis. PCR fragments used in further manipulations were isolated from 0.7% agarose gels run in Tris-borate-EDTA buffer (33). Desired fragments were purified by a phenol-free-thaw method followed by ethanol precipitation (33).

**UV and MMS sensitivity measurements.** Samples (0.1 ml) of exponentially growing cells at a 10 ^ 7 dilution were spread on LB or BHI agar. The cells were then irradiated with increasing doses of UV with the Stratalinker 2400 (Stratagene, La Jolla, Calif.). Plates were incubated at 37°C for 24 h and examined for growth. For methyl methanesulfonate (MMS) sensitivity experiments, samples (0.1 ml) of exponentially growing cells at a 10 ^ 8 dilution were plated on LB agar containing various concentrations of MMS (Sigma, St. Louis, Mo.). Plates were incubated at 37°C for 24 h and examined for growth.

**Mutagenesis of the cloned *P. gingivalis* recA homolog.** pFLL23 was constructed by subcloning a 1.9-kb EcoRI-PstI fragment carrying the recA homolog into pUC19 digested with EcoRI and PstI. The ermF-ermAM cassette was purified from pVA2198 digested with EcoRI and BamHI (11) and treated with Klenow to fill in the single-stranded ends. pFLL23 was linearized at a unique HindIII site located within codon 147 of the recA open reading frame and ligated with the *ermF-ermAM* cassette (11). After transformation into *E. coli* DH5α and selection on erythromycin (300 μg/ml) plates, a single colony (FLL24) was chosen for further study.

**Electroporation of *P. gingivalis.*** Electroporation of cells was performed as previously reported (11). One milliliter of an actively growing culture of *P. gingivalis* was used to inoculate 10 ml of BHI broth supplemented with hemin and vitamin K and vitamin K and incubated at 37°C. Seventy milliliters of the overnight incubated culture and incubated at an additional 4 h. The cells were harvested by centrifugation at 2,600 × g for 7 min at 4°C and washed in 70 ml of electroporation buffer (10% glycerol and 1 mM MgCl₂ filtered sterilized and stored at 4°C), and the pellet was suspended in 0.5 ml of electroporation buffer. A 100-μl sample of cells, to which 1 μg of DNA was added, was placed in a sterile electrode cuvette (0.2-mm gap). The cells were pulsed with a Bio-Rad gene pulser for 4.2 ms at 2,500 V (12.5 kV/cm) and then incubated on ice for 3 min. The cell suspension was then used for the electroporation. A 100-μl sample of plasmid DNA (10 μg/ml) was added to the plasmid DNA and incubated at 2,600 V (12.5 kV/cm) and then was incubated overnight at 37°C.

**Virulence testing.** *P. gingivalis* W83 and the mutant strain FLL33 were tested for virulence as described by Sambrook et al. (33). One milliliter of an actively growing culture of *P. gingivalis* was used to inoculate 15 ml of tryptic soy broth supplemented with hemin (1 μg/ml), vitamin K (1 μg/ml), and dithiothreitol (0.5 μg/ml), which was then incubated overnight at 37°C. Eighty-five milliliters of warmed medium (37°C) was then inoculated with 15 ml of the overnight culture and incubated for an additional 4 h. This culture was then used to inoculate 900 ml of tryptic soy broth, and the mixture was incubated for 24 h at 37°C. The cells were centrifuged, washed in sterile phosphate-buffered saline (0.147 M NaCl, 0.01 M sodium phosphate [PBS]) under anaerobic conditions, and adjusted to the desired concentration in PBS. All mice were challenged by subcutaneous injections of 0.1 ml of bacterial suspension at two sites on the dorsal surface. Mice were then examined daily to assess their general health status, as well as the presence and location of lesion. Body weights were determined only for all surviving mice. These experiments were performed under authorization of an institutionally approved animal use protocol (20).

**Nucleotide sequence accession number.** The nucleotide sequence reported here may be found under GenBank accession no. U70054.

## RESULTS

**Cloning of the recA homolog from *P. gingivalis* W83.** PCR was used to amplify a 320-bp fragment of the recA sequence of *P. gingivalis* W83. This PCR fragment was 5′ labeled and used to screen a DASH recombinant phage bank of *P. gingivalis* W83 genomic DNA (10) for the presence of hybridizing clones. Ten of 1.0 × 10⁸ phage clone plaques (1.0%) hybridized with the probe.

The hybridizing phage plaques were amplified and absorbed onto maltose-grown *E. coli* DH5α cells. DNA from the phage clones was isolated with the Promega Lambda Wizard DNA purification system. NotI-BamHI cleavage of purified DNA from two of the recombinants (L2 and L10) revealed that the phage clones had different restriction fragment patterns (data not shown), although L2 and L10 each contained a 2.1-kb fragment (data not shown). L2 also contained additional fragments of 8.0 and 6.5 kb, in contrast to L10, which contained fragments of 11.5, 8.3, and 0.3 kb. These data indicate that these were independent clones and not siblings from a single cloning event. L10 was chosen for further study.

**Southern blot hybridization with the 32P-labeled 0.3-fragment of the recA gene used as a probe identified the hybridizing fragment.** Plasmid pUC19 was used to subclone a 2.1-kb hybridizing BamHI fragment from L10 (designated pFLL26).

**RecA nucleotide sequence.** Both strands of the 2.1-kb BamHI fragment carried on pFLL26 were sequenced, and one 1.02 kb open reading frame corresponding previously reported for genomic *P. gingivalis* DNA (36). No SOS box (39) was found in the 5′ upstream region of the *P. gingivalis* recA homolog. A comparison of the amino acid sequence of this gene with the National Center for Biotechnology Information genetic sequence data bank revealed the highest similarities, of approximately 90, 86, and 82%, to the RecA proteins from *Bacteroides fragilis*, *Prevotella ruminicola*, and *Mycobacterium smegmatis*, respectively (Fig. 2). Furthermore, regions from amino acids 68 to 81 and 266 to 288 revealed conserved ATP binding domains (14, 17, 38).

**Complementation of an *E. coli* recA-deficient mutation by the *P. gingivalis* recA homolog.** *E. coli* HB101 transformed with plasmids carrying the recA gene from *P. gingivalis* was irradiated with increasing doses of UV (Table 1). HB101 strains carrying pFLL26 and pFLL23 (with the intact recA gene in the opposite orientation to that of pFLL26) were more resistant than the wild-type strain to UV exposure. Further, UV sensitivity was similar to the level of the wild-type HB101 strain in cells that carried pFLL24, in which the recA gene was interrupted with the *ermF-ermAM* cassette (Table 1). On LB medium containing MMS, only HB101 cells that carried the intact recA gene were able to grow at a concentration of >0.04% (Table 1).

**Construction of a recA-defective mutant in *P. gingivalis* W83.** An isogenic recA-defective mutant of *P. gingivalis* W83 was constructed by allelic exchange mutagenesis (Fig. 3). The nucleotide sequence of the cloned recA fragment revealed a unique *HinI* restriction site at bp 435 of the open reading frame. To utilize this site, a 1.8 kb EcoRI-PstI fragment containing the intact recA gene was subcloned into EcoRI-PstI-cleaved pUC19. The resulting plasmid, pFLL23, was digested with EcoRI and ligated with the 2.1-kb *ermF-ermAM* cassette from pVA2298. The recombinant plasmid, pFLL24, was used as donor DNA in electroproporation of *P. gingivalis* W83. Since the plasmid was unable to replicate in *P. gingivalis*, clindamycin-resistant transformants could arise only as a result of an integration into the wild-type gene on the chromosome. We
predicted that two double crossover events between the regions flanking the \textit{erm} marker and the wild-type gene on the chromosome would result in replacement of a segment of the wild-type gene with a fragment conferring clindamycin resistance.

Following electroporation and plating on selective medium, we detected 15 clindamycin-resistant colonies after a 7-day incubation period. These colonies were replica plated onto selective medium and exposed to UV to determine their sensitivity to UV radiation.

FIG. 2. A comparison of amino acid sequences of the \textit{P. gingivalis} W83 RecA homolog and other RecA proteins of procaryotic origin. Uppercase letters indicate conserved amino acids and/or amino acids with conservative replacements according to the following groupings: ILVM, PAGST, HKR, and QNED. Abbreviations: Pg, \textit{P. gingivalis} W83 RecA homolog; Bf, \textit{B. fragilis}; Pr, \textit{P. ruminicola}; Ms, \textit{M. smegmatis}.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Plasmid} & \textbf{Phenotype} & \textbf{MIC of MMS (\%)} & \textbf{UV radiation dose (\mu J) for 0% survival} \\
\hline
\textit{None} & \textit{RecA} & & \leq 500 \\
\textit{pUC19} & \textit{RecA} & & \leq 500 \\
p\textit{FLL26} & \textit{RecA} & > 0.04 & \leq 2,000 \\
p\textit{FLL23} & \textit{RecA} & > 0.04 & \leq 2,000 \\
p\textit{FLL24} & \textit{RecA} & & \leq 500 \\
\hline
\end{tabular}
\caption{Complementation of the \textit{E. coli} HB101 \textit{recA} mutation with the plasmid carrying the \textit{P. gingivalis recA} homolog}
\end{table}

\footnote{MMS sensitivity was determined by spreading exponentially growing cells on LB plates containing appropriate concentrations of MMS (0.001 to 0.04\%). UV sensitivity was determined by spreading exponentially growing cells on LB plates and then subjecting them to irradiation with increasing doses (0, 500, 1,000, 2,000, and 4,000 \(\mu J\)) of UV with the Stratalinker 2400 (Stratagene).}

FIG. 1. Nucleotide sequence of the \textit{recA} gene and its flanking regions from \textit{P. gingivalis} W83. The deduced amino acid sequence of the open reading frame is indicated below the nucleotide sequences. Potential procaryotic promoter sequences, as well as a possible Shine-Dalgarno (SD) sequence, are underlined. The stop codon (TAG) is indicated by an asterisk. Sequences which served as primer-binding sites for PCR amplification are in underlined boldface.

\textbf{TABLE 1. Complementation of the \textit{E. coli} HB101 \textit{recA} mutation with the plasmid carrying the \textit{P. gingivalis recA} homolog}
sitivity. Four UV-sensitive colonies were chosen from the unexposed plate for further study. To confirm the presence of the ermF-ermAM cassette in the predicted location, chromosomal DNA from the transformants was probed with 32P-labeled pFLL26 and pVA2198. If the DNA was digested with BamHI, a predicted 2.1-kb fragment should have been seen in the wild-type. Since the ermF-ermAM cassette is missing a BamHI site, a 4.2-kb fragment should have been seen in the clindamycin-resistant mutants. As shown in Fig. 4A, the predicted 2.1-kb fragment was seen in W83 (lane A). A 4.2-kb fragment was present in the clindamycin-resistant mutants of W83 (lanes B to E) when probed with 32P-labeled pFLL26, which carried the recA gene. A similar blot probed with pVA2198, which carried the ermF-ermAM cassette, revealed an identical 4.2-kb hybridizing fragment present only in the clindamycin-resistant mutants (Fig. 4B, lanes B to E). No hybridizing sequences were seen in W83 (lane A). pUC19 vector sequences did not hybridize with W83 or any of the clindamycin-resistant mutants (data not shown). These data indicate that the predicted recombination had occurred, resulting in the wild-type recA gene being interrupted by the ermF-ermAM cassette.

Characterization of P. gingivalis W83 recA mutants. recA mutants plated on brucella blood agar plates (Anaerobic Systems Inc., San Jose, Calif.) displayed phenotypes similar to the wild-type strain, which was beta-hemolytic and black pigmented (data not shown). One strain from this group, designated FLL33, was chosen for further study. A generation time of 3 h was determined for W83, in contrast to 3.5 h for FLL33. To reconfirm the loss of activity of the P. gingivalis RecA protein, we assayed the sensitivities of the wild-type and recA strains to UV irradiation. There was 80% survival of the wild-type strain, W83, to 1,000 μJ of UV irradiation, in contrast to 18% survival for FLL33. At 2,000 μJ there was 40% survival of the wild-type cells, compared to 0% survival for the recA-defective mutant. These data indicate that the recA gene plays an important role in P. gingivalis W83 survival following DNA damage by UV irradiation.

Virulence testing of FLL33 in a mouse model. At 24 h, two of five mice challenged with P. gingivalis W83 (wild type) at a dose of \(10^{10}\) bacteria/animal died. Surviving animals appeared cachectic and hunched, with ruffled hair. Although the mice did not display lesions at the dorsal surface site of injection, all had developed spreading ulcerative abdominal lesions. By 48 h, all animals had died. When mice were challenged with a similar dose of strain FLL33 (recA mutant), one of five died after 24 h. Surviving animals appeared cachectic and hunched, with ruffled hair. None of the mice in this group displayed any lesions at the dorsal surface site of injection but all had developed spreading ulcerative abdominal lesions. Three of the surviving mice had died by 48 h, and the fourth had died by day 4.

When mice were challenged with W83 at a dose of \(5 \times 10^9\) bacteria/animal, one of five died and four of five developed secondary-site lesions after 24 h. By 48 h, three of five mice in this group had died, and by day 4 only one of five survived. All had developed ulcerated abdominal lesions. The lesion in the surviving animal was resolving at day 14. In a challenge with a similar dose of FLL33, one of six mice died and five of six mice developed secondary-site lesions after 24 h. By 48 h, three of six mice in this group had died, and by day five only one of six survived. All had developed ulcerated abdominal lesions. The lesion in the surviving animal was resolving at day 14.

DISCUSSION

The degenerate oligonucleotide primers designed to amplify a conserved region in gram-positive bacteria (9) are functional in P. gingivalis. Further, two conserved ATP binding motifs were observed in the deduced P. gingivalis RecA protein, which agrees with the evolutionary conservation of this motif across bacterial species (16). In addition, the deduced P. gingivalis RecA protein shows strong similarity to RecA proteins from other gram-negative anaerobes (14). Similar to the RecA pro-

![FIG. 3. Construction of a site-specific mutant by allelic exchange. pFLL24 contained the recA gene interrupted by an ermF-ermAM cassette (ermF confers Emr in P. gingivalis, and ermAM confers Emr in E. coli [11]). The plasmid was introduced into P. gingivalis W83 by electroporation. A reciprocal recombination event between areas of homology on the target cell’s chromosome and regions flanking the Emr cassette of pFLL24 replaced the recA gene with a fragment containing ermF-ermAM. P. gingivalis cells were incubated for about 16 h post-electroporation and plated on supplemented BHI agar with clindamycin (0.5 μg/ml) for 7 to 10 days at 37°C. The cloning vector used was pUC19.

![FIG. 4. Southern blot analysis of allelic exchange mutants of P. gingivalis. Total cellular DNA from P. gingivalis was cleaved with BamHI, electrophoresed through 0.7% agarose, and bidirectionally transferred to nitrocellulose. (A) Lane A, W83; lanes B to E, allelic exchange mutants. The probe used was 32P-labeled pFLL23, which carries the P. gingivalis recA homolog. (B) Lanes are as described for panel A. The probe used was 32P-labeled pVA2198, which carries the ermF-ermAM cassette. A 4.2-kb fragment was detected in all strains which contain the recA homolog inactivated with ermF-ermAM.
tens from these gram-negative anaerobes, no consensus LexA binding sequence (CTG'TN3CAG) was present in the predicted promoter region of recA, which is in contrast to many SOS-regulated recA genes (39). This suggests that the predicted LexA homolog may bind a different sequence in the promoter region or that the P. gingivalis recA gene may not be regulated by a LexA-like protein. The recA genes from B. fragilis and Thiothrix fermentoxidans, which also lack an SOS box, are not induced by DNA damage (13, 28), in contrast to E. coli, in which that gene, as part of the SOS regulon, is induced when the cell contains DNA damage (39). Alternatively, it is possible that the P. gingivalis recA gene is not part of an E. coli-like SOS regulon. There are other cloned recA genes (14, 28) that lack identifiable LexA binding sites whose regulation is unknown, and they may represent a class of non-SOS-regulated recA genes.

The RecA protein is functionally conserved in bacteria (16). The ability of the recA homolog from P. gingivalis to complement the recA mutation in E. coli suggests functional conservation of the RecA protein from P. gingivalis. Further, expression of the recA gene was independent of any E. coli promoters. Insertional inactivation of the recA gene with the ermF-ermAM cassette significantly reduced the ability for DNA repair in both P. gingivalis and E. coli.

Strain FLL33 showed a virulence profile similar to the wild-type, W83, which is consistent with a previous report (29). Strain FLL33 confirms that the recA gene in P. gingivalis W83 plays an important role in DNA repair; however, inactivation of this gene did not affect the virulence potential of P. gingivalis in our mouse model. A possible explanation for this phenomenon would be an ability of P. gingivalis to affect the normal function of polymorphonuclear leukocytes (PMNs), thus limiting or inhibiting oxidative stress. Consistent with this hypothesis, it has been demonstrated that P. gingivalis W83 can inhibit the induction of superoxide production in human PMNs (27). Further, the LPS from P. gingivalis can prevent the production of inflammatory mediators in the mouse model (30) and inhibit E-selectin expression and neutrophil adhesion, two components necessary for the recruitment of leukocytes in response to infection (7). It is possible, therefore, that recA may be important for the survival of P. gingivalis in an oral environment, with its occasional exposure to air, or in a mixed infection, where the normal function of PMNs could be induced by the presence of other bacteria. It is believed that the persistent colonization of bacteria on the tooth root surface is responsible for the inflammatory response characteristic of periodontal disease. As a late colonizer of the tooth root surface (18), P. gingivalis could be exposed to oxidative stress due to the host response to the accumulation of the early colonizers; thus, the recA gene also may be important for DNA repair in vivo. We are investigating this further.

We have constructed the first recA-defective strain of P. gingivalis by allelic exchange mutagenesis. This represents an important step that will facilitate genetic studies (e.g., complementation analysis) for elucidating the precise role of several virulence factors in the pathogenicity of P. gingivalis. It is also possible that the recA mutation may assist in the construction of whole-cell vaccine or replacement therapy strains or in the production of subunit vaccinogens with P. gingivalis.

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