The present study evaluated the prevalence of Porphyromonas gingivalis and the correlation between the bacterial culture method and the detection of immunoglobulin A (IgA) specific to the P. gingivalis fimbrial antigen in gingival crevicular fluid (GCF). P. gingivalis was isolated from 78.3% of subgingival plaque samples obtained from active sites and 34.7% of those from inactive sites of periodontal patients. P. gingivalis was isolated from only 4.7% of healthy subjects (control group). Immunoglobulins specific to the P. gingivalis fimbrial antigen were detected by enzyme-linked immunosorbent assay (ELISA). The overall agreement between the results of the P. gingivalis culture method and the results of specific IgA detection in periodontal patients was 71.7% for active sites and 58.7% for inactive sites. IgA specific to P. gingivalis was absent in GCF from all of the sites of healthy subjects. The results suggest that P. gingivalis is associated with the local production of specific IgA. The detection of IgA antibodies specific to P. gingivalis in GCF by ELISA may be used as a predictive parameter to reveal the early phase of the activation of recurrent periodontal infections.

Materials and Methods

Selection of Patients. Thirty-three patients with severe periodontal disease were enrolled from the patients attending the Clinic of Dentistry, Section of Parodontology, of the University of Catania. Periodontal patients were selected if they had severe adult periodontitis with a pocket depth of greater than 5 mm (active site) and at least one inactive site. The patients had no history of periodontal treatment or antibiotic therapy during the previous 6 months. Twenty-one healthy subjects with no periodontal pathology were included in this study as a control group.

Collection of Subgingival Plaque and Microbiological Monitoring. Samples were obtained from periodontal pockets after supragingival plaque had been removed from the teeth to be sampled. Forty-six subgingival plaque samples were collected from periodontal patients, in particular, one sample from a single active site from each of 20 patients and one sample from each of two different active sites of the remaining 13 patients. Samples from inactive sites were collected in the same way from the same patients. A single sample was obtained from each of the 21 healthy subjects.

The subgingival plaque samples were inoculated into 2 ml of brucella broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.4-µl/ml vitamin K₃ (Sigma Chemical Co., St. Louis, Mo.) and 5-µg/ml hemin (Sigma Chemical Co.). They were then diluted and plated onto trypticase soy agar (BBL Microbiology Systems) supplemented with 10% defibrinated horse blood (Unipath, Rome, Italy), 5-µg/ml hemin, and 0.4-µl/ml vitamin K₃. The plates were incubated in duplicate in an anaerobic atmosphere for 7 to 10 days or in air plus 10% CO₂ for 2 to 4 days. The bacteria grown were selected on the basis of...
size, color, shape, and staining. The anaerobic bacteria were identified by the API 20 and rapid ID 32A (BioMerieux a La Balme, Les Grottes, France) biochemical tests and by gas chromatographic analysis (Perkin-Elmer Instrument) of fatty acids (6). Black-pigmented, anaerobic, gram-negative rods were submitted to a fluorescence test by longwave UV light; absence of fluorescence was considered a rapid taxonomic test to distinguish between P. gingivalis and other black-pigmented, anaerobic, gram-negative rods (18).

**GCF collection and serum sampling.** GCF samples were taken from at least one active and one inactive site from each periodontal patient and from one site of each healthy subject. A filter paper cone (Johnson and Johnson, East Windsor, United Kingdom) was introduced into the gingival crevice until the cone absorbed 0.5 μl of GCF (3). Cones were stored at −20°C in 100 μl of phosphate-buffered saline (pH 7.4, 0.05% Tween 20 (UV light buffer) until tested. To evaluate the presence of specific antibodies in GCF, the paper cones were eluted by shaking for 30 min at room temperature. A serum sample was obtained from each patient for detection of IgA specific to the P. gingivalis fimbrial antigen. Positive and negative serum samples and GCF specimens were selected from our collection by immunofluorescence assay and used as reference specimens.

**Fimbrial antigen preparation.** Preparation of the fimbrial antigen from P. gingivalis 381 was performed by using the modified procedure described by Lee et al. (9).

Protein concentration was evaluated by the method of Lowry et al. (12). The purity of the 43-kDa fimbrial protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with minor modifications of the Laemmli method (7). The crude fimbrial antigen was stored in aliquots at −80°C until used.

IgA and IgG determination in GCF and serum samples. The presence of IgA and IgG antibodies specific to the P. gingivalis 381 fimbrial antigen in GCF and serum samples was determined by enzyme-linked immunosorbent micro assay. Polyethylene microriter strips (Nunc Maxisorp; Nunc, Roskilde, Denmark) were coated with P. gingivalis fimbrial antigen in 0.1 M sodium carbonate-bicarbonate buffer (pH 9.6) by overnight incubation at room temperature. After washing steps, twofold dilutions of each serum sample, from 1:25 to 1:800, in phosphate-buffered saline containing 0.5% bovine serum albumin (Sigma Chemical Co.) and 0.5% Tween 20 (sample buffer) were added. GCF specimens or dilutions of serum were added to the wells, and the strips were incubated for 1 h at 37°C. Plates were washed, and peroxidase-conjugated rabbit immunoglobulins to human IgA or IgG (Dako, Glostrup, Denmark) diluted 1:3,000 and 1:15,000, respectively, in sample buffer were added. The plates were then incubated for 1 h at 37°C. After washing steps, 2,2-phenylenediamine in sodium citrate-phosphate buffer (pH 5.0) containing 0.03% hydrogen peroxide was added. Strips were incubated for 30 min at room temperature in the dark, and the reaction was stopped by adding 150 μl of 1 M HCl per well. Optical density at 492 nm was measured by a Multiskan MCC spectrophotometer (Labsystem, Helsinki, Finland).

Specimens were considered positive if their absorbance values were higher than the mean of negative controls plus 3 standard deviations (cutoff); in the case of a cutoff lower than 0.200, this value was considered to be the new cutoff.

**Statistical analysis.** Statistical studies were carried out by using the chi-square test.

**RESULTS**

**Microbiological results.** P. gingivalis was isolated from 36 (78.3%) of 46 samples obtained from active sites of periodontal patients and from 16 (34.7%) of 46 samples from inactive sites of the same patients. P. gingivalis was detected in only one specimen (4.7%) from the 21 healthy subjects.

**Immunological results.** The isolation of P. gingivalis was related to the presence of specific IgA in the GCF of the periodontal patients. In particular, 27 (58.7%) of the 46 GCF samples from active sites of periodontal patients were positive for IgA specific to the antigen tested, while only 11 (23.9%) of the 46 inactive sites of the same patients were IgA positive. Of the 46 active sites assayed, 19 (41.3%) were IgA negative, while 35 (76.1%) of the inactive sites were IgA negative.

The overall agreement of both methods for periodontal patients, calculated as the concordance of the isolation results and the IgA detection results, was 65.2% (60 of 92); in particular, the agreement was 71.7% (33 of 46) and 88.6% (27 of 47) for active and inactive sites, respectively. Concerning the discrepant results for both active and inactive sites, 25% (23 of 92) were negative for the detection of specific IgA and positive for the isolation of P. gingivalis and 9.7% (9 of 92) were positive for specific IgA and negative for bacterial isolation.

The positive predictive value of the IgA detection assay (27 [71.1%] of 38) was higher than that of the culture method (36 [69.2%] of 52).

No IgA specific to the P. gingivalis fimbrial antigen was detected in the GCF of the 21 healthy subjects, and P. gingivalis was isolated from only 1 of those subjects.

The statistical analysis carried out to evaluate the relationship between the results obtained by comparing the isolation method and specific IgA antibody detection were significant at P < 0.01 for the active sites of both periodontal patients and healthy subjects, while for the inactive sites of periodontal patients, the same relationship was significant at P < 0.05.

The titers of IgG antibody to the P. gingivalis fimbrial antigen in serum were similar for all of the subjects studied.

**DISCUSSION**

This study indicates that P. gingivalis is one of the major pathogens in the active sites of periodontal patients, since it was isolated from more than 78% of the total sites examined. Different percentages of P. gingivalis isolation in recurrent pockets have been reported (3, 4, 19, 20, 29), and these could be due to the different phases of the examination of the bacterial flora. In the early phase of periodontal disease, a high level of P. gingivalis presence is detected, while in a later stage, the recurrent pockets may become chronic and they can be colonized by other periodontopathic bacteria, leading to changes in microbial ecology (3).

Many researchers have observed an association between P. gingivalis isolation and detection of immunoglobulins, such as specific IgG, especially of the IgG4 subclass, and IgA, in the serum of adults with periodontitis (5, 8, 13, 14, 17, 25).

In this study, the P. gingivalis antigen used for IgG and IgA detection was the purified fimbrial antigen instead of the bacterial lysate antigens employed by others (17, 25). The use of the 43-kDa fimbrial antigen should enhance the specificity of the serological method, avoiding the cross-reactions that often occur when bacterial cell extracts are employed in serology. Even though the antigen used in this study was different, our results support those of Schenck et al. (17), indicating no statistically significant differences in the levels of IgG antibodies to P. gingivalis detected in serum samples obtained from periodontal patients and healthy subjects. One explanation for this observation could be that since P. gingivalis belongs to the normal oral microflora (22), the humoral IgG is often present in both healthy subjects and periodontal patients. We supposed that a better indication of active P. gingivalis colonization of the gingival pockets would be a different class of immunoglobulins that can be produced at the site of infection. Many investigators have, in fact, detected antibodies specific to periodontal pathogens in GCF and saliva, suggesting local immunoglobulin production by gingival cells at active periodontal sites (2, 3, 15, 17, 21). Since IgA is one of the earliest immunological responses to infection, especially at a site of infection, our interest was to study the relationship between P. gingivalis isolation and a specific IgA response in GCF sampled at sites of periodontal infection.

The analysis of our results indicates that detection of IgA specific to the P. gingivalis fimbrial antigen in GCF is a useful tool for the determination of bacterial colonization of gingival pockets. In 71.7%, in the majority of the active-site samples, P. gingivalis was isolated and it was significantly correlated with the detection of specific IgA in GCF. Moreover, this correlation is also confirmed because in 80% of the cases in which P. gingivalis was not isolated, IgA was not detectable. These results were also supported by those obtained with samples from
in both inactive and active sites of periodontal patients. At these sites, in fact, both isolation and IgA detection were negative in 76.6% of the cases and in only a few healthy sites was P. gingivalis isolated and correlated with the presence of specific IgA. The agreement between the culture method and the serological method, in both active and inactive sites of periodontal patients, confirms the specificity of the IgA method. Since in many cases, one inactive and one active site were sampled from the same tooth, it can be supposed that the bacteria colonize a unique niche of the tooth and the immune response is particularly strong only at the site of colonization.

These results were further confirmed in the group of healthy subjects. In this group, the agreement (95.2%) between the absence of P. gingivalis and the absence of a specific local immune response indicates that specific activation of a local IgA response is frequently present in the course of active P. gingivalis infection.

As regards the discrepant results obtained for both the active and the inactive sites of periodontal patients, where P. gingivalis was isolated in the absence of specific IgA detection, it can be supposed that contamination of the subgingival specimens by the supragingival microflora occurred during sampling because P. gingivalis is also usually present in saliva and in the oral microflora (16, 22). On the contrary, the few cases of discrepant results in which IgA was detected in the absence of bacterial isolation could be due to inactivation of P. gingivalis during transport of the subgingival plaque samples to the laboratory. Another explanation for this last result could be inactivity of the culture procedure to detect the target organisms, especially in single subgingival plaque samples taken from periodontally diseased sites (10).

The present study suggests that P. gingivalis, which is one of the most important periodontal microorganisms in the early stages of recurrent periodontal disease, is associated with local production of specific IgA. Although the effective role of local IgA in GCF is still unclear, it probably protects gingival tissues against the destructive effects of the periodontopathic bacterial flora, modulating the phase of recurrent periodontal infections.

The presence of specific IgA in these specimens could be a parameter indicating an early phase of periodontal infection. In fact, the immune production of specific IgA in this phase of the disease probably occurs at an early stage of active P. gingivalis colonization of the dental pocket.

Studies are still in progress to confirm this hypothesis. If it is confirmed, the detection of specific IgA antibodies in GCF by enzyme-linked immunosorbent assay may be useful as a predictive parameter to reveal the early phase of the activation of recurrent periodontal infection and to suggest to the periodontist the type and frequency of specific antibacterial periodontal treatment. Therapy could be more effective if it were applied in the early phase of periodontal disease.

The easy sampling of GCF, the absence of any contamination of the sample by interfering factors, the simple way of detecting IgA compared with interfering methods, and the good correlation between specific IgA detection and P. gingivalis colonization make this immunological method an appropriate diagnostic tool for the determination of recurrent periodontal disease.

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