Isolation of Porphyromonas gingivalis and Detection of Immunoglobulin A Specific to Fimbrial Antigen in Gingival Crevicular Fluid

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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.

Porphyromonas gingivalis has been frequently isolated in several oral diseases, including pulpal infections, oral abscesses, and periodontitis (11, 26). Cells of anaerobic, gram-negative, and black-pigmented bacteria can be the predominant periodontopathic bacteria in recurrent infections of adult periodontitis. The first pathogenic step involves microbial colonization, and P. gingivalis has been shown to adhere to epithelial cells, salivary proteins, and other oral bacteria by using filamentous surface appendages or fimbriae. Although this mechanism is still not completely understood, several observations (28) suggest that the fimbriate or fimbria-like structures play an important role in the adhesion of the bacteria to the tooth or oral epithelial surfaces. Lee et al. (9) have shown that monoclonal antibodies to purified fimbriae and synthetic peptides analogous to the fimbriation sequence block the adherence of P. gingivalis to oral epithelial cells and to oral surfaces.

The local virulence factors of P. gingivalis do not seem to have a direct effect on bone resorption but stimulate the production of numerous inflammatory cytokines that are able to increase osteoclastic activation. The effect of osteoclastic cells is represented by the damage caused by epithelial attachment, destruction of collagen, and alveolar bone resorption (20). Gingivitis and periodontal diseases also stimulate the local and systemic immune reactions mediated by B cells.

While inflammatory cytokines are thought to be associated with the principal lesions of periodontitis, the humoral immune system might play a role in the mediation of the development of gingivitis and periodontitis, as many investigators have demonstrated (23, 24). The concentrations of immunoglobulins specific to whole bacterial cells, to lipopolysaccharide, and to the fimbrial protein of different periodontopathic bacteria are high in patients with adult periodontitis compared to those in healthy subjects (1, 14, 17, 25). The fimbriae are really a species-specific component of P. gingivalis and are a useful tool for determining human antibody response (27). The specificity of fimbrial antigen is important for detection of a specific immune response because the N-terminal amino acid sequence of fimbriin has been shown to be completely different from those of fimbriins of other bacteria, including Bac teroides nodosus, Escherichia coli, Neisseria gonorrhoeae, Moraxella nonliquefaciens, and Pseudomonas aeruginosa (28).

The purpose of this study was to evaluate the prevalence of P. gingivalis in subgingival plaque samples and the correlation between culture methods and detection of immunoglobulin A (IgA) specific to P. gingivalis fimbrial antigen in gingival crevicular fluid (GCF) of patients with acute recurrent periodontitis. This correlation may lead to the employment of the IgA parameter as a prognostic tool for local P. gingivalis activation. Moreover, the titers of humoral IgG specific to the same antigen were also investigated.

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 Periodontology, 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 each of the 20 active sites and one sample from each of the 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 K1 (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 K1. 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...
For specific IgA and negative for bacterial isolation. For the isolation of 92 were negative for the detection of specific IgA and positive in particular, the agreement was 71.7% (33 of 46) and 58.6% (27 of 46) and the IgA detection results were 65.2% (60 of 92); in particular, 35 (76.1%) of the inactive sites were IgA negative.

Determination of IgA specific to the antigen tested, while only 11 (23.9%) of samples from active sites of periodontal patients were positive in periodontal patients. In particular, 27 (58.7%) of the 46 GCF related to the presence of specific IgA in the GCF of 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 lyase 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 Schenk 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 fact, 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 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 inability of the culture procedure to detect the target organism, 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 culture 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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REFERENCES


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