Double-Blind Analysis of the Relation between Adult Periodontitis and Systemic Host Response to Suspected Periodontal Pathogens

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By using a sensitive enzyme-linked immunosorbent assay, 200 randomly selected sera from Red Cross blood donors were screened for immunoglobulin G (IgG), IgA, and IgM levels against Actinobacillus actinomycetemcomitans, Bacteroides gingivalis, and Bacteroides intermedius. A subgroup of 79 blood donors was clinically examined for type and extent of periodontal destruction, and serological and clinical data were subjected in all possible dual combinations to correlation analyses. The results revealed that the majority of the blood donors suffered from moderate to severe adult periodontitis, often coupled with severe gingival inflammation. No cases of localized juvenile periodontitis or rapidly progressive periodontal destruction were encountered. The extent of periodontal destruction proved to be significantly correlated only to the IgG response levels against B. gingivalis. Corresponding correlation tests assessing the relationships of loss of attachment, bone loss, pocket depth, and papillary bleeding index with the IgG responses to A. actinomycetemcomitans were of marginal significance, while the IgG responses to B. intermedius revealed no relationship to the periodontal health status. The specific IgM responses proved to be unrelated to the clinical parameters, but interestingly, they were found to be highly correlated with each other. Specific IgA levels were frequently too low for enzyme-linked immunosorbent assay testing and, therefore, had to be exempted from statistical analyses. Assessments of the serotype specificity of strongly elevated IgG responses to A. actinomycetemcomitans disclosed no evidence for an association of a particular serotype-specific IgG response with the occurrence of adult periodontal destruction. In contrast to results of earlier studies, a number of sera were found to contain strongly elevated IgG levels against two or even all three serotypes. Although derived by an alternative approach, the reported results largely corroborate earlier observations linking only the occurrence of elevated anti-B. gingivalis IgG responses to the presence of marked periodontal lesions in adults.

Periodontal destruction is a common feature of a variety of clinically distinct diseases that are likely to have a specific, though possibly complex, microbial etiology (see references 26, 28, and 33 for reviews). However, the finding that bacteria which predominate at sites of existing periodontal destruction are also encountered at quiescent sites (29) implies that periodontal destruction is the consequence of a disequilibitation within the subgingival microflora and between the subgingival microflora and the host defense system. The functions and mechanisms of the host response to bacterial colonization and invasion of periodontal structures have been the subject of continuous discussions (see reference 10 for a review). Numerous local and systemic host responses, both antigen-specific and nonspecific, have been recognized. Nevertheless, their concerted role in the pathogenesis of periodontal diseases remains poorly understood.

Many recent studies concentrated on the analysis of the systemic host response to suspected periodontal pathogens, hoping to gain further insight into the microbial etiology of periodontal diseases. Moreover, an unambiguous association of specifically elevated antibody titers with active periodontal destruction could have important diagnostic implications (24). Overall, a pronounced positive association between specific antibody levels in serum and the occurrence of periodontal destruction was observed, although conflicting data have been reported as well (4, 10). A particularly high degree of association has been described between the presence of antibodies against Actinobacillus actinomycetemcomitans and the occurrence of localized juvenile periodontitis. Patients with adult or with rapidly progressive periodontitis revealed elevated antibody titers against this organism much less frequently (6, 7, 11, 15, 25, 30, 32), but were reported to have often significantly increased immunoglobulin G (IgG) levels to Bacteroides gingivalis (18, 21, 23, 30, 31). On the other hand, IgG levels to Bacteroides intermedius or to spirochetes, which are both suspected of participating in the pathogenesis of adult and rapidly progressive periodontitis (16, 26, 29, 35), were never found to differ significantly between patient and control groups (17, 21, 23, 30).

The purpose of this study was to assess a randomly selected adult population for its serum antibody reactivity patterns with A. actinomycetemcomitans, B. gingivalis, and B. intermedius. We also wanted to test whether the observed host responses are correlated with the occurrence and extent of periodontal destruction. The three bacterial species were chosen because of their predominance in the microflora of periodontal lesions and their capacity to destroy, at least in vitro, components of periodontal structures or the host defense system (see reference 26 for a review). Instead of comparing the serology of clinically defined patients and control groups, we followed a double-blind approach that allowed a complementary evaluation of the results and conclusions of previous studies.

MATERIALS AND METHODS

Sera. Plasma from 200 blood donors, randomly selected and coded, were provided by the Swiss Red Cross. The samples were supplemented with sodium azide to a final

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concentration of 0.01% and stored in aliquots at −20°C. Blood
samples of the Swiss Red Cross are unpaid volunteers.

**Bacterial antigens.** *A. actinomycetemcomitans* A370, 511, Y-4, and NCTC 9710, representing serotypes a, a, b, and c, respectively (36); *B. intermedius* ATCC 25611 and ATCC 25261, representing genotypes I and II (14, 34), respectively; and *B. gingivalis* 381 were used in this study. Strain *A. actinomycetemcomitans* A370 was obtained from G. Pulverer, University of Cologne, Cologne, Federal Republic of Germany; while strains Y-4, 511, and 381 were received from S. S. Socransky, The Forsyth Dental Center, Boston, Mass. All strains were grown in fluid medium (12), further supplemented for the growth of *B. gingivalis* 381 with 1% heat-inactivated horse serum. *A. actinomycetemcomitans* was grown in an atmosphere of 10% CO₂ in air, whereas *B. intermedius* and *B. gingivalis* cultures were incubated anaerobically in jars (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 2 and 7 days, respectively.

For the assessment of IgG and IgA titers, a protein-enriched bacterial cell supernatant (BCS) (2) was used. This had the advantage that all experiments could be performed with a single bacterial antigen preparation. Extensive pre-testing, as noted above, showed that antibody levels of these two isotypes did not differ when assessed against intact bacteria or BCS. To account for the heterogeneity among *A. actinomycetemcomitans* strains (36), BCS of strains A370, Y-4, and NCTC 9710 were adjusted to identical protein concentrations and then mixed in equal parts. The heterogeneity of *B. intermedius* (12–14, 34) was considered by using an analogously prepared mixture of BCS from strains ATCC 25611 and ATCC 25261.

IgM titers had to be determined against intact bacteria, as the reactivity with BCS was too low. Mixtures of the *A. actinomycetemcomitans* and *B. intermedius* isolates mentioned above were prepared by combining equal parts of bacterial suspensions which had been previously adjusted to an optical density at 550 nm of 0.5.

**Experimental approach of the serological analysis.** Levels of IgG, IgA, and IgM in serum against the three bacterial species were measured by an enzyme-linked immunosorbent assay (ELISA). Both the experimental design and the transformation of the data into ELISA units (EU) are based on the observation that the dilution profiles of the sera consistently resulted in sigmoidal curves with a range of linear decline. Regression lines drawn through the linear range of the dilution profiles had the same slope, if the same antigen and the same enzyme-conjugated second reagent were used. Thus, antibody levels could be assessed at single dilutions, and data from test sera could be directly compared with those of a reference serum, provided that (i) the test sera were analyzed at concentrations within the linear range, (ii) the reference serum was included on the ELISA-microtest plate with a dilution profile, and (iii) this profile displayed the regular slope.

Hence, we first selected a reference serum for each antibody response type to be assessed. All reference sera were chosen to have an intermediate antibody titer. To get well-defined test conditions, we assayed each of the 200 sera at least once at intermediate serum dilutions. Then, the specific antibody levels of all sera were measured by two to five additional experiments, each performed in triplicate at the predetermined optimal dilutions. IgM titers were tested at a serum dilution of 1:100, while the dilutions for IgG determinations had to be varied between 1:300 and 1:18,000. The IgA measurements were performed at dilutions between 1:25 and 1:200.

**ELISA.** A modification of the indirect ELISA technique of Engvall and Perlmann (9) was used as described previously (12). For antigen coating, ELISA microtiter plates (Peta Plastic) were incubated with excess amounts of BCS or bacteria. For quantification of human IgA binding, horseradish peroxidase-conjugated goat anti-human IgA (Fc) (Cappel Laboratories, Cochranville, Pa.) was used at a dilution of 1:1,000. Specific IgG and IgM levels were assessed with horseradish peroxidase-conjugated goat anti-human IgG (Fc) (Cappel) and goat anti-human IgM (Fc) (1:1,000) sera (Nordic, respectively). The extent of antibody binding in ELISA was determined spectrophotometrically at 492 nm with a Titertek Multiscan (Flow Laboratories Ltd.). All ELISA data are expressed in EU, derived by comparison of the ELISA activity of the test serum with the corresponding activity of the reference serum, which was arbitrarily defined as 1,000 EU. To calculate the EU of a test serum, the ratio of the logarithms of the EU of test and reference sera (log EUₜ and log EUₖ, respectively) was made equal to the ratio of the two ELISA-defined parameters bₜ and bₖ: log EUₜ/log EUₖ = bₜ/bₖ. Parameter bₖ corresponds to the y-axis intersection of the regression line obtained from the dilution profile of the reference serum, while bₜ is the y-axis intersection determined by shifting the reference serum regression line through the data point of the test serum. For calculation of EUₜ, the equation given above was transformed to EUₜ = EUₖbₖ/bₚ.

**Subgroup of blood donors for clinical analysis.** For technical reasons a clinical examination of all blood donors was not feasible. We selected a subgroup of blood donors with the aim of obtaining a representation of the whole study group that was as good as possible and of covering the entire spectrum of observed antibody reactivity patterns. To this end, IgG, IgM, and IgA titers were grouped according to high, intermediate, or low antibody levels against each of the bacterial antigens. Then, six lists with all 162 possible triple combinations were established, and each serum sample was assigned on each list to the titer combination that corresponded to its particular reactivity pattern. From each combination at least three sera (or if the combination contained less than three sera, all of them) were selected, and the 108 donors were called by the Swiss Red Cross for clinical examination.

We are aware that this selection process was nonrandom and thus was not in agreement with requirements for the subsequent statistical analyses. However, apart from an eventuality slightly preferred selection of rare antibody reactivity patterns, the process could be expected to yield a representative subgroup. It was devised solely to enhance the chances to examine clinically the few potentially very interesting blood donors with extreme antibody response patterns.

**Clinical examination.** Of the 108 blood donors that were contacted, 79 responded and were examined by a clinical investigator not yet involved in the study. The volunteers were analyzed by standard procedures (20) for loss of attachment; pocket depth; bone loss (determined interdentally at six sites per quadrant by measuring on radiographs the distance from the cementoenamel junction to the alveolar bone crest); papillary bleeding index; number of teeth; tooth mobility; number of decayed, missing, or filled surfaces (DMFS); and number of overhangs.

Statistical analysis. Relations among the isotype-specific antibody responses of all 200 blood donors were investigated by the Pearson moment-product correlation coefficient analysis. To estimate the normality of the antibody titre fre...
frequency distributions, the data were tested against a normal distribution by the Kolmogorov-Smirnov test. Sex dependence of specific antibody levels was evaluated by the Student t test. Relations between serological and clinical parameters were assessed in all possible dual combinations by the Pearson moment-product correlation coefficient analysis. All statistical calculations were performed with the Statistical Analysis System computer programs (SAS Institute Inc., Cary, N.C.).

RESULTS

Relations among the observed antibody responses. In Fig. 1A and B are shown the frequency distribution diagrams of all IgG and IgM levels against A. actinomycetemcomitans, B. gingivalis, and B. intermedius. The IgM titer frequencies proved to be normally distributed ($P > 0.02$), provided that logarithmically transformed EU were used for analysis. Among the IgG titers, those to A. actinomycetemcomitans and B. intermedius showed approximately normal frequency distributions, while the distribution of the anti-B. gingivalis responses was non-normal.

Specific IgA levels mostly were found to be very low. In fact titers of only about 20% of all sera could be determined by ELISA for a particular IgA response, as even dilutions of 1:25 proved to be insufficient to obtain ELISA readings within the linear range of the dilution profile. Therefore, only elevated IgA responses were determined (see below), and no IgA data were considered for statistical analyses.

In search of possible relations among the specific antibody responses of a particular isotype and of eventual relationships between the different antibody responses to the same bacterial species, all IgG and IgM titers were subjected in all possible dual combinations to correlation analyses. Interestingly, the IgM responses of the blood donors to the three bacterial species showed highly significant correlations with each other ($P = 0.0001$). The Pearson moment-product correlation coefficients ($r$) were between 0.759 and 0.773, indicating that more than 50% ($r^2 > 0.5$) of the total variation can be explained by the variation of one variable. This finding demonstrates an unexpected and close relationship between the three IgM responses. The IgG responses correlated with each other as well ($P < 0.01$), but the extent of relationship between the variables was much smaller ($r$ between 0.180 and 0.310). When the respective IgG and IgM responses were analyzed for eventual correlations, clearly insignificant results were obtained ($0.143 < P < 0.610$).

Sex and age dependence of the observed antibody levels. The 200 Red Cross sera were donated by 105 females and 95 males. No relationship could be found between the sex of the blood donors and their specific IgG responses. The IgM levels were consistently higher among females, but statistical significance was demonstrated only in the case of the IgM
responses to A. actinomycetemcomitans (P < 0.02 by the Student t test; the two other P values were 0.027 and 0.105).

The age of the blood donors varied from 19 to 67 years; both mean and median were at about 44 years. Males and females showed very similar age distributions. While the IgM response levels to the three bacteria proved to be negatively correlated to the age of the blood donors (P < 0.01; r between −0.248 and −0.301), the IgG responses to A. actinomycetemcomitans and B. gingivalis (Fig. 2), but not those to B. intermedium, revealed significant positive correlations with donor age. However, the coefficients of determination (r²) were below 0.1.

Composition of the clinical subgroup. The clinical subgroup contained 48 females and 31 males with age distributions nearly identical to those of the entire blood donor group. The distributions of the specific antibody levels observed in the two groups were also very similar (Fig. 1), as only sera with high IgG levels against A. actinomycetemcomitans or B. gingivalis appeared to be over-represented in the clinical subgroup. Based on these data, a statistical analysis of the results obtained with the clinical subgroup seemed warranted.

The periodontal health status of the 79 clinically examined blood donors is shown in Fig. 3. It describes the prevalence of both gingival inflammation and periodontal destruction, as the mean papillary bleeding index was used as an indicator of the degree of gingival inflammation, while the severity of periodontal destruction was demonstrated by the product of the mean loss of attachment (LA) and the mean pocket depth (PD). The choice of this product has the advantage that overrating of severe gingival swellings or of large gingival retractions, which both may erroneously indicate periodontal destruction, is reduced. The data suggest that the clinically analyzed blood donors could be divided roughly into three groups with different severities of adult periodontitis. The first group with a small LA × PD product (between 0.5 and 3.4) contained individuals with gingivitis or mild adult periodontitis, as defined by Page and Schroeder (22). Only 28% of these blood donors had periodontal pockets of 5 to 6 mm in depth. The members of the second group suffered from moderate adult periodontitis; 82% of the persons had pockets of at least 5 mm in depth, and 33% displayed predominantly local pocket formation of 7 or more mm in depth. The papillary bleeding indices were mostly

FIG. 2. IgG levels in serum reactive with B. gingivalis plotted versus the age of the serum donors. Each point of the scatter diagram represents data from one blood donor (N = 200).

FIG. 3. Relation between periodontal destruction and gingival inflammation. Each point represents the data from one individual. The extent of periodontal destruction is shown by the product LA × PD. The level of gingival inflammation is indicated by the papillary bleeding index (PBI). Regions A and B comprise blood donors with moderate and severe adult periodontitis, respectively. Similarly, the regions labeled I, II, and III indicate blood donor groups with increasing severity of gingivitis.
between 1.5 and 3, so the vast majority of these persons simultaneously had gingivitis and adult periodontitis. The third group, with an LA × PD value of >9, comprised individuals with severe adult periodontitis accompanied in most cases by severe gingivitis. These blood donors had experienced severe vertical bone loss; 88% had at least one pocket of 7 or more mm in depth; most, however, had several sites with pockets of 7 or more mm in depth. Notably, neither cases of localized juvenile periodontitis nor of rapidly progressive periodontitis (22) were observed.

Correlation analyses of serological and clinical data. Six serological variables, namely the IgG and IgM levels against A. actinomycetemcomitans, B. gingivalis, and B. intermedius, and nine clinical variables (mean loss of attachment, mean bone loss, mean pocket depth, maximum pocket depth, number of pockets deeper than 7 mm, papillary bleeding index, number of overhangs, DMFS, and number of teeth) were considered for correlation analyses. Whole-mouth scores were used, with the exception of mean bone loss, in which only data from six sites per quadrant were available. Overall, data from 78 blood donors were tested in all possible dual combinations. One blood donor was excluded from the analyses because the patient suffered from extraordinarily severe periodontal destruction which possibly would have immediately influenced the statistics.

The IgG levels to B. gingivalis were found to be significantly correlated (P < 0.02) with loss of attachment, bone loss, and pocket depth (Table 1). The respective coefficients of determination were small (r² < 0.2) and suggested a limited relevance of these correlations. But because of the skewed frequency distribution of the IgG levels against B. gingivalis, this observation should be judged cautiously. Further measures thus were taken to evaluate the relationship between attachment loss and anti-B. gingivalis response. In Fig. 4 is shown a scatter diagram, with the mean loss of attachment scores plotted versus the IgG levels against B. gingivalis. A definite relationship between the variables is recognizable, although the presence of both clearly elevated antibody levels (>3 log EU) among individuals with less than 2-mm mean attachment loss and low IgG titers among blood donors with 3- to 5-mm mean loss of attachment is apparent. If a 2 × 2 contingency table was construed with these data by using cutoff levels of <2-mm loss of attachment and >3.0 log EU IgG anti-B. gingivalis, cells of 11 (<2 mm, >3 log EU), 29 (>2 mm, >3 log EU), 22 (<2 mm, <3 log EU), and 16 (>2 mm, <3 log EU) data points were obtained. These results indicate an association of elevated IgG responses to B. gingivalis with evident loss of attachment at a sensitivity of 73%, and thus, suggest a considerable relationship between the variables. The specificity of the test reached 58%.

In contrast to the significant correlations between the various periodontal disease parameters and the IgG responses to B. gingivalis, neither the DMFS scores nor the number of overhangs revealed a relationship with this part of the host response (Table 1). Furthermore, none of the

![Graph](http://iai.asm.org/)

**Fig. 4.** Relationship between the IgG levels in serum against B. gingivalis and the mean loss of attachment of the blood donors. Each point represents the data from one individual. The variables are significantly correlated, but the coefficient of determination is low (P = 0.0007; r² = 0.143).
clinical parameters correlated with the IgM levels against B. gingivalis (P values >0.05; −0.245 < r < 0.189).

The correlation tests assessing the relationship between the periodontal parameters and the IgG levels against A. actinomycetemcomitans are listed in Table 2. The data suggest a definite relationship between the variables, as the correlations reached statistical significance in one case (P < 0.02), while in four other cases, correlations were at the border of significance. However, the relevance of these correlations should be regarded as low, because the coefficients of determination did not reach 0.1. The IgM levels specific for A. actinomycetemcomitans were found to be unrelated to the various clinical parameters. None of the respective P values was below 0.1.

Finally, no correlations were detected between the periodontal disease parameters and the IgG responses to B. intermedius (Table 3). Even the association of the IgG levels with the papillary bleeding index, which is often used as a descriptor of the extent of acute gingival inflammation, was too weak to reach significance. Evaluation of scatter diagrams produced by these correlation tests demonstrated a particular high frequency of high-titered serum IgG responses among blood donors with little or no periodontal destruction.

**Elevated IgA responses.** Titers of sera with clearly elevated IgA responses to at least one of the three bacterial species used (i.e., those that could be analyzed at dilutions of 1:25 or more) were determined in additional experiments, and the data were plotted versus the LA × PD product of the respective blood donors (Fig. 5). The figure gives no indications for significant relationships between these IgA levels and the extent of periodontal destruction. Apparently, highly elevated IgA responses against any of the three bacterial species could be encountered among individuals with very mild periodontitis or gingivitis, as well as among severely diseased blood donors.

**Serotype specificity of elevated IgG responses against A. actinomycetemcomitans.** Following the completion of the correlation analyses, 33 sera from clinically examined individuals with high IgG titers against A. actinomycetemcomitans (>1,000 EU) were analyzed further for A. actinomycetemcomitans serotype-specific IgG levels. The data are shown in Fig. 6, with the sera arranged according to the LA × PD product of their donors. This product is shown together with the respective papillary bleeding indices and the maximum pocket depths of the blood donors to allow an estimation of the periodontal health status of the donors. Although made with intact bacteria of the strains 511 (serotype a), Y-4 (serotype b), and NCTC 9710 (serotype c), the tests corroborated the presence of high IgG levels against A. actinomycetemcomitans in all these sera. However, they provided no indications for an eventual association of serotype-specific IgG levels with the extent of periodontal destruction in the adult. Most sera reacted predominantly with one serotype. Reactivity with the serotype b isolate was particularly pronounced. Interestingly, though, several sera revealed more complicated reactivity patterns, and in fact, all possible combinations of simultaneously elevated IgG levels to two or all three serotypes could be detected among the 33 sera. This is in contrast to previous observations (36), in which the reactivity of human sera with only a single A. actinomycetemcomitans serotype was described.

**DISCUSSION**

In this study we sought to assess randomly selected blood donors for their antibody response patterns to suspected periodontal pathogens and to analyze the relationship between the specific antibody levels and the extent of periodontal destruction. To our knowledge, this approach is in contrast with previous studies, as it required neither the often biased selection of patient and control groups nor the definition of cutoff levels to distinguish between elevated and background antibody responses. On the other hand, the approach has the disadvantage that it emphasizes linear relationships between the antibody response levels and the severity of periodontal destruction and that it requires scores

<table>
<thead>
<tr>
<th>TABLE 2. Correlation analyses between IgG anti-A. actinomycetemcomitans levels and various clinical parametersa</th>
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<td>Clinical parameters</td>
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<tr>
<td>Loss of attachment</td>
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<td>Bone loss</td>
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<td>Pocket depth</td>
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<td>Maximum pocket depth</td>
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<td>Papillary bleeding index</td>
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<td>Number of overhangs</td>
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<td>DMFS</td>
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a See footnotes a and b in Table 1.

**TABLE 3. Correlation analyses between IgG anti-B. intermedius and various clinical parameters**

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>No. of blood donors</th>
<th>Correlation coefficient</th>
<th>P value</th>
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<tbody>
<tr>
<td>Loss of attachment</td>
<td>77</td>
<td>0.190</td>
<td>0.0975</td>
</tr>
<tr>
<td>Bone loss</td>
<td>60</td>
<td>0.158</td>
<td>0.2273</td>
</tr>
<tr>
<td>Pocket depth</td>
<td>78</td>
<td>0.162</td>
<td>0.1563</td>
</tr>
<tr>
<td>Maximum pocket depth</td>
<td>78</td>
<td>0.106</td>
<td>0.3567</td>
</tr>
<tr>
<td>Papillary bleeding index</td>
<td>78</td>
<td>0.205</td>
<td>0.0714</td>
</tr>
<tr>
<td>Number of overhangs</td>
<td>78</td>
<td>0.106</td>
<td>0.3626</td>
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<tr>
<td>DMFS</td>
<td>78</td>
<td>0.043</td>
<td>0.7114</td>
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a See footnotes a and b in Table 1.

FIG. 5. IgA levels in serum against A. actinomycetemcomitans (A), B. intermedius (B), and B. gingivalis (C) plotted versus the LA × PD product. The specific IgA titers of most sera were below the sensitivity of the assay. The horizontal line in each panel indicates the limit of detectability. The points represent the few sera with strongly elevated IgA titers against the respective bacterial antigens.
FIG. 6. IgG levels in serum reactive with the three A. actinomycetemcomitans serotypes a (striped columns in the front), b (white center columns), and c (dotted columns in the back). Thirty-three high-titered sera from members of the clinical subgroup were considered. The sera are arranged according to the LA × PD product of their donors. The papillary bleeding index (PBI) and the probing depth of the deepest pocket (MAX PD; in millimeters) in the dentition of the blood donors are also given to enable a rough estimation of the degree of respective periodontal destruction. The value of 1 kEU (1,000 EU) corresponds to an optical density in ELISA of 0.8, with the serum diluted 1:2,000.

from the whole mouth, unless many more blood donors could be analyzed. The linear relationship might be limited as long lasting host responses could possibly be triggered during initial, local periodontal destruction. Nevertheless, if the specific host response and the occurrence of adult periodontitis are indeed significantly related, both approaches should reveal this clearly, and therefore, the present study should be suited for a complementary evaluation of previous findings.

In fact, the IgG responses to B. gingivalis and the principal periodontal disease parameters were found to be quite significantly correlated with each other. In spite of the
impossibility of precisely describing the extent of the relationship detected by the correlation tests, our results agree with those of previous studies that suggested a distinct association of the humoral immune response to \textit{B. gingivalis} with adult periodontitis (8, 19, 21, 30, 31). This interpretation of the correlation tests is supported by the data obtained from the contingency table analysis, as the results were well within the range of previous observations (8, 19, 30, 31). The rather low specificity of the analysis is explained in part by the frequent occurrence of low-titered IgG anti-\textit{B. gingivalis} responses among apparently diseased blood donors. As discussed previously by Mouton et al. (18), these cases could indicate a heterogeneous microbial etiology of adult periodontitis or may reflect the influence of immune suppression. On the other hand, 10\% of the clinically examined blood donors revealed clearly elevated responses to \textit{B. gingivalis} (>3.2 log EU), in spite of the fact that they had only 2 mm or less of attachment loss. Inspection of the site-specific raw data collected during clinical examination showed that seven of these eight blood donors had, nevertheless, one or two gingival pockets of at least 5 mm in depth that became masked by the scores from the whole mouth. Thus, the presence of these pockets could explain the development of the observed elevated antibody titers (because of the double-blind approach, microbiological sampling at these sites was not possible), and there is no evidence from the results of this study that there were truly donors with false-positive results, namely periodontally healthy individuals with high IgG levels against \textit{B. gingivalis}.

\textit{A. actinomycetemcomitans} and the humoral host response to this organism have been associated with localized juvenile and rapidly progressive periodontitis rather than with adult periodontal disease (5-8, 10, 15, 22, 25, 30, 32). Nevertheless, an increased frequency of sera with high titers against \textit{A. actinomycetemcomitans} has repeatedly been observed in patient groups with adult periodontitis (8, 21, 30). This latter aspect is clearly confirmed by results of the present study, as correlation tests probing the relationship between the periodontal parameters and the IgG levels to \textit{A. actinomycetemcomitans} nearly reached statistical significance, and a contingency table analysis (data not shown) revealed virtually identical cells as the one discussed for the anti-\textit{B. gingivalis} responses. Nevertheless, a strong association between the IgG titers to \textit{A. actinomycetemcomitans} and the occurrence of adult periodontitis may not be inferred from our results. According to the observed coefficients of determination, the relevances of these correlations (which were much less influenced by a skewed titer frequency distribution than those against \textit{B. gingivalis}) were very low, and several high-titered sera were apparently derived from blood donors with gingivitis but no gingival pockets. Observations similar to the latter have been reported by Zambon et al. (36) and Naito et al. (21), whereas in other studies (8, 30) frequencies of elevated anti-\textit{A. actinomycetemcomitans} responses below 1.5\% have been described. Our results do not contradict a possible etiologic role of \textit{A. actinomycetemcomitans} in adult periodontitis but suggest that these antibody responses result at least in part from stimulation during gingivitis or from extroral challenges.

Similar conclusions must be drawn to explain the observed lack of relationship between the periodontal disease parameters and the IgG responses to \textit{B. intermedia}. This result is not surprising because the species is known to be part of the microflora of gingivitis (27) and to colonize also nonoral mucous surfaces (1, 35). The result is in good agreement with observations made by others who reported failure to detect significant differences between the frequencies of elevated antibody responses to \textit{B. intermedia} in control and patient groups (21, 23, 30).

When dealing with \textit{A. actinomycetemcomitans} and \textit{B. intermedia}, it should be considered that both are antigenically heterogeneous (3, 13, 19, 36). Up to now only isolates of \textit{A. actinomycetemcomitans} have been described to be consistently separable into distinct serogroups by polyclonal antisera (36\%). Zambon et al. (36) reported that strains of serotypes a and b could be isolated with equal frequency from healthy individuals or from patients with adult periodontitis, while serotype c was encountered less frequently.

In contrast, isolates from patients with localized juvenile periodontitis were described to belong predominantly to serotype b (36). By assessing data from selected blood donors with high titers against \textit{A. actinomycetemcomitans}, we could not detect any evidence for a preferential association of one of the serotypes with the occurrence of adult periodontitis. Moreover, several sera reacted strongly with two or even all three serotypes. Apparently, this has not been observed among the sera studied by Zambon et al. (36) and indicates that multiple infections with several serotypes may occur. Certainly, our results cannot be explained by antibody interaction with cross-reactive antigens from \textit{A. actinomycetemcomitans} serotypes or from \textit{A. actinomycetemcomitans} and some closely related \textit{Haemophilus} species. First, such cross-reactivities could not have revealed the described heterogeneous antibody-binding patterns; second, immunoblots (data not shown) revealed that the serum antibodies were predominantly directed against a major serotype-specific antigen.

The most prominent phenomena encountered in this study were the strong correlations among the IgM responses of the blood donors to the three bacterial species. The observation may be explained in at least three different ways: (i) the bacterial species share common antigenic determinants recognized by the IgM antibodies; (ii) many of the sera contained significant amounts of rheumatoid factors; and (iii) the sera contained natural species-specific IgM antibodies which, as the result of a known regulatory mechanisms, occurred consistently in the same proportions, although their concentrations varied widely from individual to individual. Only further studies may help to answer these questions, although results of preliminary immunoblot experiments with electrophoretically separated bacterial antigens and some high-titered sera from this study have indicated that these IgM antibodies are indeed directed against species-specific antigens (C. Wyss and B. Guggenheim, unpublished data), thus supporting the third hypothesis.

In conclusion, in spite of the use of an alternative approach, our results largely corroborate earlier findings associating the host response to \textit{B. gingivalis} and, to a lesser extent, to \textit{A. actinomycetemcomitans} with the occurrence of periodontal destruction in adults. Further longitudinal studies assessing variations of the host response in relation to the presence or absence of locally active periodontal destruction are required for a better evaluation of an eventual serodiagnostic potential of host response screening.

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LITERATURE CITED


