

Humoral Immunity of Older Adults with Periodontal Disease to *Porphyromonas gingivalis*

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The effect of age on the humoral response to *Porphyromonas gingivalis* was assessed in groups of adults (25 to 54 years and 55 to 74 years) with periodontal disease and compared with that in age-matched healthy controls. To determine whether there was an antibody response against *P. gingivalis*, we measured serum antibodies against whole cells of *P. gingivalis* 381, A7A1-28, and W50. In addition, antibody levels against purified *P. gingivalis* outer membrane proteins (i.e., the 43-kDa fimbrial protein and a 75-kDa protein) were also evaluated. Elderly subjects showed the same response to *P. gingivalis* as younger subjects. Immunoglobulin G (IgG) antibodies to both purified proteins were also elevated in both diseased groups as compared with the normal groups. Total serum IgG, IgA, and IgM levels were also determined by an enzyme-linked immunosorbent assay for all four groups. Total serum IgG levels were elevated in older adults with periodontitis and total IgA levels were elevated in both groups of older adults compared with the younger groups of similar disease status. Total serum IgM levels were comparable for the four groups. Antinuclear antibody titers were assessed in the two groups of older adults and were also found to be higher for the group with periodontitis. These studies show that older adults as well as younger adults have markedly elevated specific antibodies of the IgG and IgA classes to antigens of *P. gingivalis*, a putative pathogen in both groups. Furthermore, older adults with periodontitis have significantly elevated levels of total serum IgG which may possibly be related to higher levels of autoantibodies.

It is generally accepted that destructive periodontal disease progresses in association with a change in the microflora of the gingival pocket from a predominantly coccal gram-positive flora to a more pathogenic and anaerobic gram-negative flora (10). *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Bacteroides forsythus*, and *Campylobacter rectus*, along with several other oral bacteria, have been implicated in the initiation and progression of periodontal disease (36-38, 42; reviewed in references 40 and 49).

Patients with severe periodontal disease have been shown to have elevated levels of antibodies directed against many of the putative oral pathogens, including *P. gingivalis* (8, 28, 46). Antibodies to surface components of *P. gingivalis* have also been detected frequently in patients with periodontal disease, in particular to the 43-kDa fimbrial protein (30, 47) and to the 75-kDa major surface component (48). This organism has also been frequently isolated from active sites of periodontal disease (6, 22, 38, 44) and has also been found in the subgingival flora of older adults with periodontitis (33).

Recently, studies have focused on the identification of individuals at high risk for the development of periodontal disease so that earlier intervention can be instituted. A possible group at higher risk for more severe periodontal disease are individuals of advanced age, since the prevalence and severity of periodontal disease have been reported to increase with age (1, 17). The cause for this increase is, however, still unclear. One possibility is the well-docu-

mented decline in immune function which has been associated with aging (24). This decline has been shown to affect the cellular (34) as well as the humoral (11) branches of the immune system. While much information is available on the decline of cellular functions, the decline in the antibody response has not been fully investigated. Reported abnormalities for B cell function in the elderly include an increase in autoantibody production to a multitude of self-antigens (4, 23, 25). Increased levels of total serum immunoglobulin G (IgG) and IgA have also been a consistent finding in the elderly population (2, 25, 32). In many studies, the antibody response to specific antigens, both T cell dependent and independent, has been shown to decline (35). It is unclear whether this decline in the specific humoral response is a direct defect in B cells or is a result of defective immune system regulation. Regulatory suppression of the antibody response could be due to a decrease in helper T cell function, an increase in suppressor T cell function, or an increase in autoanti-idiotypic suppression (5, 20, 26, 43).

Since it has been speculated that the decline in the immune response is responsible in part for the increase in other infections in elderly individuals (31), we were interested in investigating the humoral immune response to periodontal pathogens in elderly subjects diagnosed with severe periodontal disease. Sera from older subjects (55 to 74 years of age) with destructive periodontal disease were compared with those from age-matched older subjects with normal periodontal tissues for total immunoglobulin levels and specific antibodies to *P. gingivalis* and other putative periodontal pathogens. These two groups of older adults were also compared with younger adults (25 to 54 years of age), both with periodontal disease and with normal periodontal tissues.

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MATERIALS AND METHODS

Human subjects. Ninety subjects were assigned to one of four groups according to age and periodontal condition. Results from the 1985–1986 National Survey of Oral Health in U.S.-employed adults and seniors indicate a more pronounced increase in the prevalence of attachment loss around the age of 60 (27). Therefore, to detect humoral factors potentially contributing to this increased attachment loss, we established 55 years of age as the cutoff between younger and older individuals. Individuals were also categorized according to periodontal status. Severe adult periodontal disease was considered present when two or more teeth had an interproximal clinical attachment loss of ≥ 6 mm (13). The periodontium was defined as healthy when all teeth exhibited an attachment loss of ≤ 3 mm. These groups consisted of older adults with healthy periodontal tissues (15 females and 7 males between the ages of 55 and 74 [mean = 62.6 ± 6.3]), older adults with periodontal disease (12 females and 17 males between the ages of 55 and 74 [mean = 65.7 ± 5.7]), younger adults with healthy periodontal tissues (8 females and 8 males between the ages of 25 and 54 [mean = 36.3 ± 5.9]), and young adults with periodontal disease (14 females and 9 males between the ages of 25 and 54 [mean = 43.9 ± 6.7]).

Microorganisms. The strains of *P. gingivalis* used in this study were obtained from the State University of New York at Buffalo culture collection. The following strains were used: 381, isolated from a subgingival plaque sample from an adult periodontitis patient (S. S. Socransky, Forsyth Dental Center, Boston, Mass.); A7A1-28, isolated from an adult periodontitis patient with non-insulin-dependent diabetes (J. J. Zambon, State University of New York at Buffalo, Buffalo, N.Y.); and W50, isolated from a clinical specimen (University of Cologne, Cologne, Germany). Bacteria were grown to the early stationary phase in half-strength brain heart infusion medium (pH 7.4) (Difco Laboratories, Detroit, Mich.) supplemented with 5 mg of yeast extract, 5 μ g of hemin per ml, and 0.2 μ g of menadione (Sigma Chemical Co., St. Louis, Mo.) per ml and incubated at 37°C under anaerobic conditions (85% N₂, 10% H₂, 5% CO₂). Cells were harvested by centrifugation at 12,000 $\times g$ for 30 min. Pellets were washed three times with 1 mM EDTA in phosphate-buffered saline (0.02 M PO₄, 0.15 M NaCl [pH 6.9]) (PBSE). Bacteria were fixed in 0.5% Formalin overnight at 4°C. Formalin-fixed cells were washed three times in PBSE and stored at -70°C until use.

Enzyme-linked immunosorbent assay (ELISA) for quantitation of total serum IgG, IgA, and IgM levels. Dynatech Immunolon II microtiter plates were coated with a 1:1,000 dilution of goat anti-human IgG, IgA, or IgM heavy chains (Calbiochem, San Diego, Calif.) in carbonate buffer (0.05 M CO₃ [pH 9.6]) and incubated overnight at 4°C. Prior to use, the plates were washed five times in a microplate washer (Bio-Rad Laboratories, Richmond, Calif.) with a saline solution (0.15 M NaCl) containing 0.5% (vol/vol) Tween 20. The plates were blocked for 1 h at room temperature (RT) with 1% bovine serum albumin (BSA) in phosphate-buffered saline (0.01 M PO₄, 0.15 M NaCl [pH 7.2]) (PBS) to prevent nonspecific binding. One hundred microliters of the appropriate dilution of test serum (1:7,500 for IgM, 1:40,000 for IgA, and 1:300,000 for IgG) was added, and the mixture was incubated at RT for 2 h. Calibrated human sera (2 \times concentrated) from Sigma were serially diluted with 1% BSA in PBS six times starting with initial dilutions of 1:2,500 for IgM, 1:10,000 for IgA, and 1:100,000 for IgG. The six dilutions

were included on each plate as reference standards. The plates were washed as described above, 100 μ l of a 1:1,000 dilution of biotinylated goat anti-human IgM, IgA, or IgG was added to each well, and the mixture was incubated for 2 h at RT. The plates were again washed, 100 μ l of a solution of alkaline phosphatase conjugated to streptavidin (Zymed Laboratories Inc., San Francisco, Calif.), diluted 1:1,000 in 1% BSA in PBS, was added to each well, and the mixture was incubated overnight at RT. After the final wash, *p*-nitrophenol disodium phosphate (Sigma) was added to carbonate buffer (0.05 M CO₃, 1 mM MgCl₂ [pH 9.8]) to a concentration of 1 mg/ml, and 200 μ l was added to each well. At the appropriate time, the reaction was stopped with 30 μ l of 2.5 N aqueous NaOH solution. The optical density of each well was determined with a model 3550 microplate reader (Bio-Rad) at 405 nm.

Individual patient sera were tested in triplicate, and a coefficient of variation not exceeding 10% was considered acceptable. The correlation coefficient of the reference sera was considered acceptable at $r = >0.99$. Values were calculated with the microplate manager software package (Bio-Rad) and are given as milligrams of immunoglobulin per milliliter of serum for each subject.

Total IgG levels in both groups of older adults, i.e., those with and those without periodontal disease, were also determined by rocket immunoelectrophoresis. Goat anti-human IgG (Sigma) diluted 1:300 with 1% agarose in Tris-barbital buffer (10 mM barbituric acid, 100 mM Tris, 0.4 mM calcium lactate, 2.5 mM sodium azide [pH 8.6]) (T-BB) was cast onto Gel-Bond film (FMC BioProducts, Rockland, Maine). Wells holding volumes of 5 μ l were cut across the middle of the film because IgG migrates towards both the anode and the cathode. A standard curve was generated by serially diluting (1:2) a calibrated human serum specimen (Sigma) six times starting with a 1:40 dilution. Individual serum specimens from the elderly subjects were diluted 1:80, and the serum concentrations were calculated from the standard curve generated on the same gel. Gels were electrophoresed at 200 V for 3 h in T-BB. Gels were washed in 0.1 M NaCl for 2 h, with a change of the washing solution every 15 min to remove unprecipitated proteins and an addition of distilled H₂O to remove the NaCl. Rockets were visualized by staining with a solution of 0.1% Coomassie brilliant blue in methanol-H₂O-glacial acetic acid (5:5:1). The rocket length, measured from the rocket tip on the cathode side to the rocket tip on the anode side for each standard dilution, was plotted against the serum concentration at that dilution. The concentrations of individual test sera were extrapolated from the standard curve according to the length of the rocket generated under the same conditions.

Quantitation of antibodies specific for whole cells of selected strains of *P. gingivalis*. Antibodies were quantified by a modified ELISA as described by Ebersole et al. (7). Wells of Dynatech Immunolon II microtiter plates were each coated with 150 μ l of formalinized whole bacteria suspended in carbonate buffer (pH 9.6) at a cell concentration of 1.3×10^{10} /ml; plates were incubated at 37°C for 3 h and stored at 4°C until use. Prior to use, the plates were blocked with a solution of 1% BSA in PBS for 1 h at RT. Three serial dilutions of test sera were made for each subject, and 100 μ l of these dilutions was added to each well. A standard curve was generated by serially diluting a positive pool which was arbitrarily assigned a value of 100 ELISA units (EU). After 2 h of incubation, the plates were washed five times with a 0.15 M solution of NaCl containing 0.05% Tween 20. Alkaline phosphatase-conjugated goat anti-human IgG, IgA, or

IgM was diluted 1:1,000, 100 μ l of these dilutions was added to each well, and the plates were again incubated for 2 h. The plates were washed as described above, and 100 μ l of substrate solution (1 mg of *p*-nitrophenyl phosphate in 0.05 M sodium carbonate–1 mM MgCl₂ [pH 9.8]) was added to each well. The reaction was allowed to proceed for 30 min, at which time 30 μ l of 2.5 N NaOH was added to stop the reaction.

Individual patient sera were tested in triplicate, and a coefficient of variation not exceeding 10% was considered acceptable. The correlation coefficient of the reference sera was considered acceptable at $r = >0.99$. EU values for test sera were calculated by multiplying the EU value calculated from the standard curve by the dilution factor.

Purification of the 43-kDa fimbrial component and the 75-kDa membrane component from *P. gingivalis*. *P. gingivalis* was grown in brain heart infusion broth for 2 days, and the 43-kDa fimbrial protein was purified by the procedure of Sojar et al. (41). In brief, cells were sonicated at a pulse setting of 20 W with a 50% duty cycle for 10 min to prepare a fimbria-rich extract. The extract was centrifuged at 100,000 $\times g$, and the supernatant was used as a source of the 43-kDa fimbrial protein and the 75-kDa membrane component. Purification was carried out by the following procedure. The 43-kDa polymer was preferentially fractionated in the presence of 1% sodium dodecyl sulfate and 0.2 M MgCl₂ at pH 6.5. The precipitate was redissolved in 50 mM Tris-HCl buffer, and the process was repeated four times, resulting in a homogeneous preparation of the 43-kDa component of fimbriae free of detectable 75-kDa component (41). The 75-kDa protein was selectively purified from the extract by repeated precipitation at pH 5.0. The 43- and 75-kDa proteins from *P. gingivalis* 2561 were purified to greater than 99% homogeneity by these methods. The purified components reacted as a single band with antisera against whole cells, as well as with specific affinity-purified polyclonal antibodies prepared against the 43- and 75-kDa components.

Absorption of sera with formalinized *P. gingivalis* whole cells. Sera from 10 subjects who showed high levels of antibody to *P. gingivalis* in an ELISA were absorbed individually with each of the three strains of *P. gingivalis* (381, A7A1-28, and W50). Each serum specimen, diluted 1:10 in PBS, was mixed with formalinized *P. gingivalis* cells that had been washed three times with PBS prior to use in the absorption experiments. The suspensions were incubated overnight at 4°C and centrifuged for 10 min to remove the bacterial cells. This procedure was repeated, and sera were tested in an ELISA for reactivity to each of the three *P. gingivalis* strains as described above.

Detection of antinuclear antibodies and rheumatoid factor. Sera from the older adults were tested for the presence of antinuclear antibodies and rheumatoid factor. The titer of antinuclear antibodies for each subject was determined by indirect immunofluorescence (Sigma Diagnostics, St. Louis, Mo.). The presence of rheumatoid factor was assessed by the Immunoscan latex agglutination test (Microscan, West Sacramento, Calif.). Both procedures were carried out in accordance with the manufacturers' instructions.

Statistical analysis. The nonparametric Mann-Whitney test was used for statistical analysis in all antibody studies. Four comparisons were made for the groups tested: (i) for younger adults, normal versus diseased; (ii) for older adults, normal versus diseased; (iii) for normal adults, young versus old; and (iv) for diseased adults, young versus old. Only the comparisons showing a significant difference are reported in the figures. To determine the interaction of the variables

(i.e., disease status, age, and gender) present in the four groups, we performed three-factor analysis of variance.

RESULTS

Quantitation of total serum immunoglobulins. Total serum IgG, IgA, and IgM levels were quantitated for the four study groups. The older periodontal disease subjects showed a significant increase in the total IgG concentration (Fig. 1) compared with both their age-matched healthy control subjects and the younger adults with periodontal disease. Both elderly groups showed an increase in the total IgA level (Fig. 1) compared with the younger groups of similar disease status. Comparison of the two younger groups revealed an elevation ($P = 0.02$) in the total serum IgA level in the group with periodontal disease, indicating a possible association between an elevated level of total IgA and periodontal disease. The elevation in the total IgA level observed in both older groups was likely to have been due to age, as reported by other investigators (2, 25, 32). However, a slightly higher elevation in the total IgA level was observed in the older diseased group as well as in the younger diseased group, but this elevation was not statistically significant. No difference in the IgM levels was observed among any of the groups tested.

To confirm the ELISA results, we quantitated total serum IgG levels in both groups of older adults by rocket immunoelectrophoresis. Both methods yielded similar results: older adults with periodontal disease exhibited a statistically significant elevation ($P < 0.005$ for ELISA; $P < 0.05$ for rocket immunoelectrophoresis) in total IgG levels compared with older adults with a normal periodontium. The mean level of IgG in milligrams per milliliter was higher for the older adults with periodontal disease when tested by the ELISA than when tested by rocket immunoelectrophoresis. This difference may have been due to different sensitivities of these procedures. However, it was quite clear that the older adults with periodontal disease showed an elevation in the total IgG level regardless of the method used. This IgG elevation is likely to be associated with periodontal disease and not age alone.

Levels of IgG antibody to *P. gingivalis*. The levels of serum IgG antibody to three strains of *P. gingivalis* were evaluated by an ELISA in older adults and younger adults with periodontal disease. Such levels were then compared with those in age-matched periodontally healthy controls. Subjects between the ages of 25 and 54 and with periodontal disease showed increased levels of IgG to all three serologic variants of *P. gingivalis* compared with subjects of similar ages but without the disease (Fig. 2). The older group with periodontitis showed similar increases in levels of IgG to *P. gingivalis* A7A1-28 and W50 but not 381. This difference in the response to strain 381 could possibly have reflected a difference in the strains of *P. gingivalis* infecting these two adult populations with periodontal disease. The data for individual subjects indicated that the majority of the subjects exhibiting elevated IgG levels responded to more than one strain of *P. gingivalis* and possibly represented a subgroup of infected or responsive individuals. Overall, these data indicate that the IgG antibody response of the elderly diseased population appears to be similar to that of younger adults with periodontal disease, i.e., exhibiting elevated levels of serum antibodies to *P. gingivalis*. In addition, similar percentages of subjects in both diseased groups (older, 41.4%; younger, 39.1%) exhibited an elevation in the level of IgG

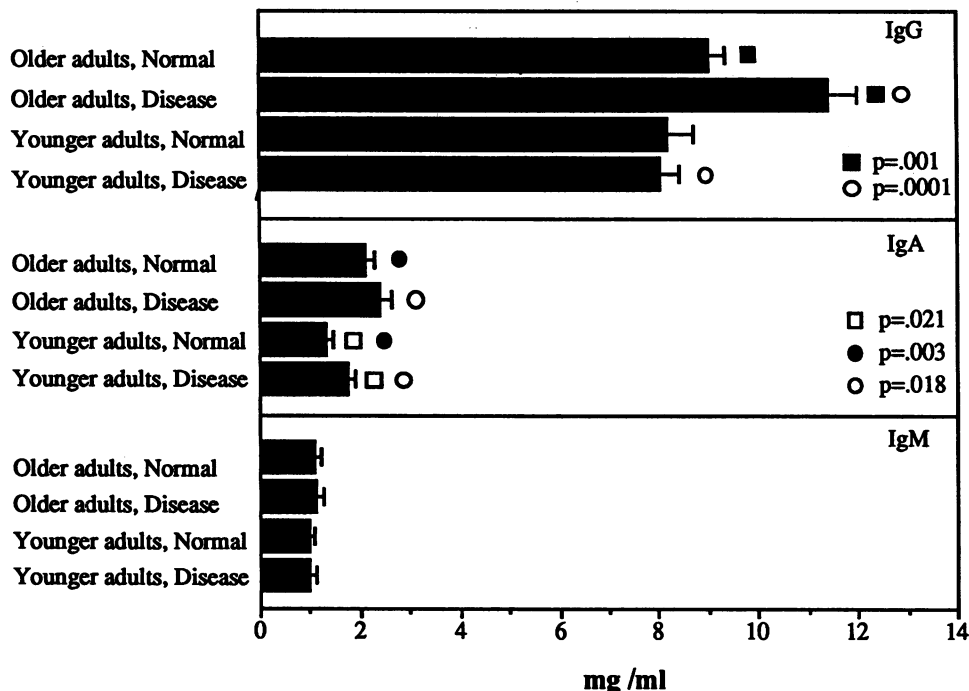


FIG. 1. Quantitation of total serum IgG, IgA, and IgM levels by an ELISA. Values represent the mean for each group \pm the standard error of the mean. Four statistical comparisons were made and are represented by the following symbols: □, younger adults, diseased versus normal; ■, older adults, diseased versus normal; ●, normal adults, younger versus older; ○, diseased adults, younger versus older. Only *P* values representing a statistical difference, determined by the Mann-Whitney rank test, are shown.

antibodies specific for at least two of the strains of *P. gingivalis* tested.

Levels of IgA and IgM antibodies to *P. gingivalis*. The levels of IgA and IgM antibodies to *P. gingivalis* were determined

by an ELISA, and the results for IgA are shown in Fig. 3. A significant increase in the level of IgA antibody specific for *P. gingivalis* 381, A7A1-28, and W50 was measured in younger patients with periodontal disease compared with

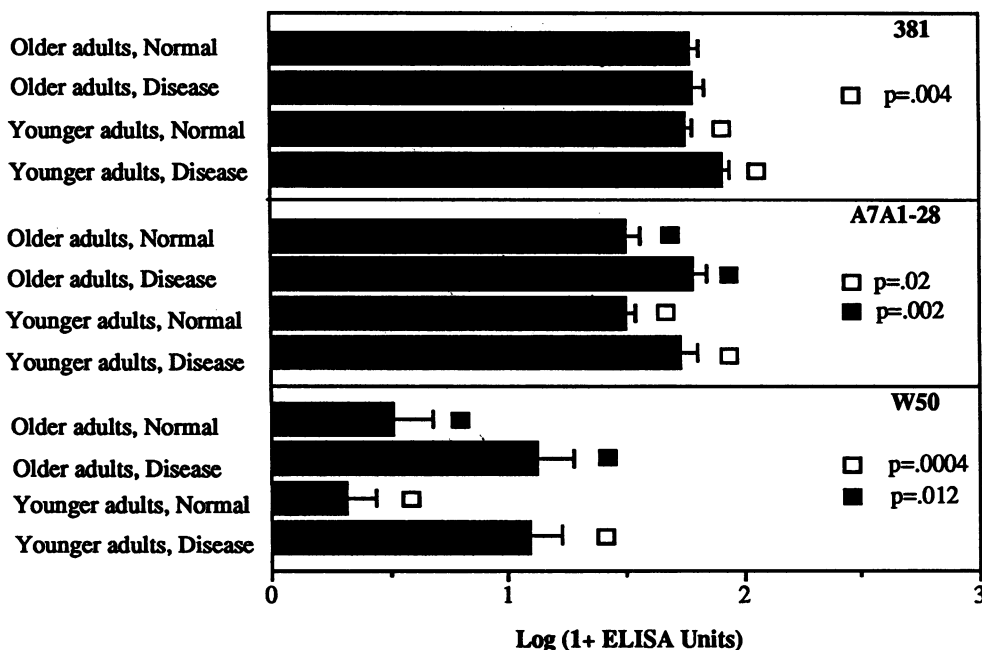


FIG. 2. IgG response against three strains of *P. gingivalis* whole cells. Values represent the mean EU for each group \pm the standard error of the mean. Symbols are as defined in the legend to Fig. 1. Only *P* values representing a statistical difference, determined by the Mann-Whitney rank test, between two groups are shown.

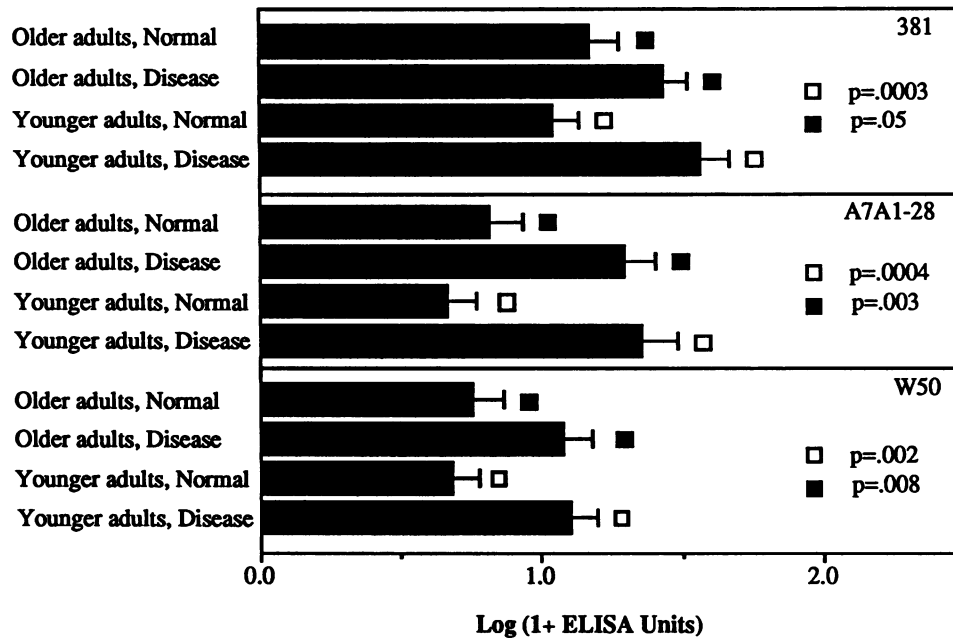


FIG. 3. IgA response against three strains of *P. gingivalis* whole cells. Values represent the mean EU for each group ± the standard error of the mean. Symbols are as defined in the legend to Fig. 1, and P values are as defined in the legend to Fig. 2.

age-matched healthy controls. A similar difference was also seen in older adults with periodontal disease, suggesting that their IgA antibody response to *P. gingivalis* was comparable to that of younger adults with periodontal disease.

No significant differences were seen in IgM levels to the three *P. gingivalis* strains, except for a slight elevation of serum IgM antibody to *P. gingivalis* W50 in the older adults with periodontal disease ($P = 0.02$) (data not shown).

Effect of gender on the level of total serum immunoglobulins and specific antibodies to *P. gingivalis*. Because of the differences in the numbers of males and females within each of the four groups, we attempted to investigate the effect of gender in these studies. Three-factor analysis of variance was performed on all data as described in Materials and Methods. Gender was not a significant factor in any of the cases in which a significant difference due to disease status was observed.

Cross-absorption of human IgG antibodies to three antigenically different *P. gingivalis* strains. To examine the cross-reactivity of human antibodies to strains 381, A7A1-28, and W50, we absorbed sera from subjects with elevated levels of IgG antibodies to *P. gingivalis* with formalized whole cells of each of the three strains and then tested them for remaining antibody activity. In all cases, a large percentage of the reactivity was removed by the absorption procedure, indicating a high level of cross-reactivity among the three strains (Table 1). However, approximately 15 to 17% of serum antibody activity directed to strains A7A1-28 and W50 could not be removed by absorption with strain 381, suggesting that strains A7A1-28 and W50 have antigens not shared by strain 381. Similarly, 22 to 27% of the antibody activity directed to strains 381 and A7A1-28 could not be removed by absorption with strain W50, suggesting that strains 381 and A7A1-28 have antigens not shared by strain W50. These experiments suggest that humans with periodontal disease have specific antibodies to *P. gingivalis* and that these antibodies react to common as well as unique antigens of *P.*

gingivalis strains, results expected for antibodies induced by specific infections with one of a series of antigenic variants of *P. gingivalis*.

Reactivity of sera from periodontal disease patients to the purified 43-kDa fimbrial component and the purified 75-kDa outer membrane antigenic component from *P. gingivalis*. Levels of serum IgG antibodies against purified antigens of *P. gingivalis* were determined for younger and older periodontal patients and compared with those for age-matched healthy controls (Fig. 4). Older and younger patients with periodontal disease showed a marked increase in the levels of IgG antibody to the 43-kDa fimbrial protein compared with age-matched adults with healthy periodontal tissues. Furthermore, both groups suffering from periodontitis (old and young) had elevated levels of serum antibodies to the purified 75-kDa outer membrane antigen. There was a good correlation between subjects with a positive IgG antibody response to whole *P. gingivalis* cells and the 43-kDa ($r = 0.66$) and 75-kDa ($r = 0.64$) antigens. Since IgG often binds nonspecifically to bacterial components, we also assessed the reactivity of human sera to the *P. gingivalis* 43- and 75-kDa components by immunoblotting. It was clear that not all human sera contained IgG reactive with either the 43-kDa

TABLE 1. Evaluation of the extent of cross-reactivity to strains of *P. gingivalis* of human serum IgG antibodies

Test strain of <i>P. gingivalis</i>	% Remaining activity ± SEM after absorption with the following strain of <i>P. gingivalis</i> ^a :		
	381	A7A1-28	W50
381	2.42 ± 0.26	17.09 ± 2.80	14.54 ± 2.56
A7A1-28	8.07 ± 4.3	0.54 ± 0.08	8.69 ± 3.44
W50	22.08 ± 6.45	27.43 ± 5.86	3.74 ± 0.09

^a Calculated by the following formula: (mean response of the absorbed sera/mean response of the unabsorbed sera) × 100.

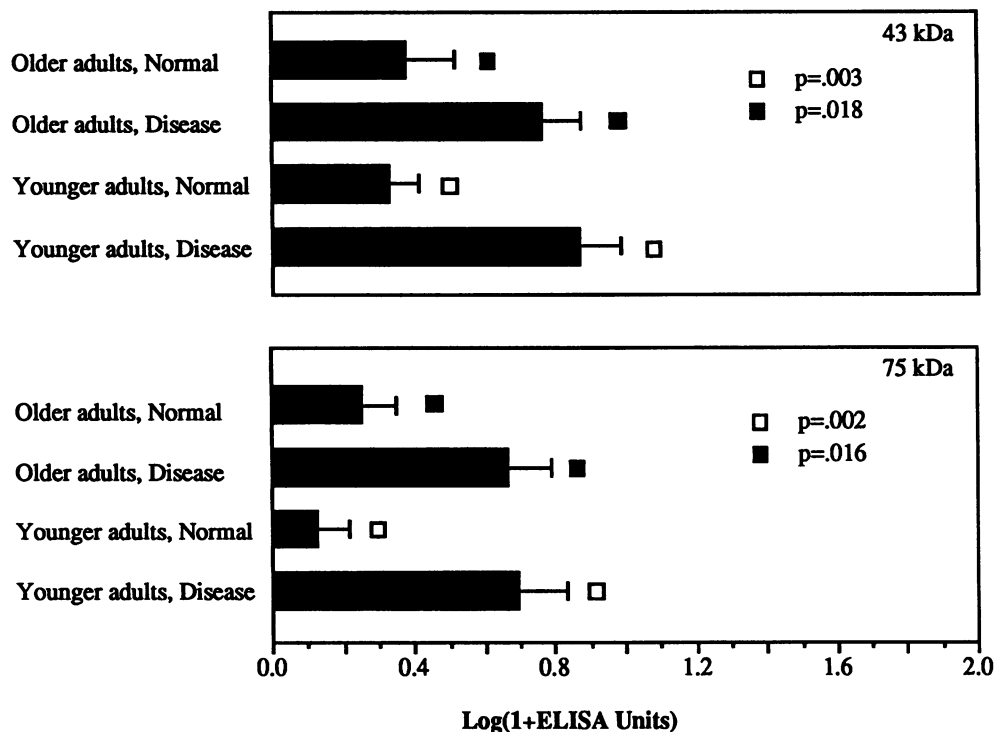


FIG. 4. IgG antibodies to purified outer membrane proteins from *P. gingivalis* 381. Values represent the mean EU for each group \pm the standard error of the mean. Symbols are as defined in the legend to Fig. 1, and *P* values are as defined in the legend to Fig. 2.

fimbrial component or the 75-kDa outer membrane component (data not shown), suggesting that the reactivity seen with human IgG was likely to be specific. Figure 4 shows that representative sera from adults with periodontitis had strong IgG reactivity with the 43- and 75-kDa components while sera from normal adults failed to react.

Detection of antinuclear antibodies in older adults. Sera from older adults were tested for the presence of antinuclear antibodies and rheumatoid factor. The percentages of subjects with detectable levels of antinuclear antibodies (64 versus 50%, respectively) and rheumatoid factor (6.9 versus 13.9%, respectively) were similar for both the diseased and the normal groups. However, the titers of antinuclear antibodies detected were higher in subjects in the diseased group. A larger percentage of diseased subjects (31%) than of normal subjects (9.1%) exhibited titers of ≥ 80 . Rheumatoid factor was detected in 3 of 22 normal subjects (titers = 20, 40, and 80) and in 2 of 29 diseased subjects (titers = 80 and 160).

DISCUSSION

This study indicates that older subjects with periodontal disease are capable of producing antibodies to *P. gingivalis* at elevated levels equivalent to those observed in younger subjects with periodontal disease. The levels of IgG, IgA, and IgM against three strains of *P. gingivalis* were assessed and determined to be significantly different between the healthy and diseased groups for each age range. However, the levels of antibodies were not different between the two diseased or healthy groups of different ages. In a companion study of these same subjects, it was found that *P. gingivalis* was a common inhabitant of the subgingival areas of both younger and older adults with periodontal disease but was

not often found in their age-matched controls with healthy periodontal tissues (33).

To further characterize the serum antibody response to *P. gingivalis*, we determined the IgG levels to putative virulence factors, i.e., the 43-kDa fimbrial antigen and the 75-kDa membrane-associated antigen, of one of the strains of *P. gingivalis* and found elevated levels of antibodies to these antigens in both groups of adults with periodontal disease. It is possible that while the quantitative antibody responses are similar in periodontitis patients, whether they are young or old, the functional capabilities of the antibodies being produced may be different, on the basis of their avidity and subclass distribution. It has been shown in other investigations that elderly subjects may produce antibodies of reduced avidity (9, 12, 29); however the significance of this observation in the function of antibodies remains to be clarified.

An interesting observation was made in tests of the total immunoglobulin levels in the four groups included in this study. Total levels of IgG and IgA were found to be elevated in the older population, a finding that many other investigators have reported (2, 14, 18, 25). In addition, we observed that older individuals with periodontal disease showed an elevation in the total IgG level that was not observed for age-matched healthy individuals. These data were obtained by two independent methods of IgG determination, ELISA and rocket immunoelectrophoresis. This elevation in the IgG level may have been a factor predisposing these subjects to periodontal disease or, alternatively, may have been a result of periodontal infection of older adults. The level of total serum IgA was significantly increased in younger subjects with periodontal disease compared with age-matched control subjects, indicating an association between increased total IgA level and periodontal disease. On the other hand, the

total IgA level in the older adults may have been primarily affected by age. Therefore, it appears that the total IgA level is associated with disease in the younger population and age in the older population.

The reported elevation in autoantibodies in the elderly population may be implicated as a cause for the elevation in IgG observed in the older population (25). The present study supports this idea in that we have also shown an increase in the titer of antinuclear antibodies in a subpopulation of older adults exhibiting an elevation in the total serum IgG level. A recent report showed an increase in the total IgG level in patients with rheumatoid arthritis as well as an increase in the incidence and severity of periodontal disease. The elevation in the IgG level was significantly correlated to alveolar bone loss in this population (45). In addition, a similarity exists between the patterns of tissue destruction seen in periodontal disease and rheumatoid arthritis (39). While a clear correlation was not observed in this study between an increase in rheumatoid factor in the older group and periodontal disease, there may have been a correlation between periodontal disease and autoimmunity, as suggested by a reported increase in autoantibodies to type I collagen in patients with severe periodontal disease (15).

Other interesting correlations which may somehow bear on the immunologic findings were observed in this study, one being the higher incidence of high blood pressure reported by subjects in the older diseased group (39%) than in the age-matched healthy group (9.5%). A correlation between the plaque index and blood pressure was reported in the 1960–1962 Health Examination Survey (19); however, no other reports showing this correlation were found. A higher percentage of subjects who had smoked for over 5 years was also observed in the diseased group of older adults as well as in the diseased group of younger adults. Although there is no clear link between the specific antibody response to *P. gingivalis* in the periodontal disease groups and these factors, they should be considered in future experiments as possible modifying influences on the immune response to periodontal organisms.

This study shows that older adults with periodontal disease exhibit an elevated serum antibody response to *P. gingivalis* whole cells and associated antigens comparable to that of younger adults with periodontal disease. Furthermore, older diseased subjects show an elevation of the total IgG level as well as an increased titer of antinuclear antibodies, which are not seen in age-matched subjects with a healthy periodontium. These elevations may be predictive of or predispose the former group to periodontal disease or may result from periodontal infection. Although the precise role of serum antibodies to *P. gingivalis* antigens is not known, it is likely that these antibodies are at least in part protective, since immunization of animals with *P. gingivalis* protects against the destructive effects of periodontal infections (3, 16, 21). Since older adults with periodontal disease mount a significant antibody response to periodontal organisms, it is clear that a reduced humoral immune response to periodontal pathogens is not likely to account for the increased prevalence and severity of periodontal disease seen in older adults.

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