Measurement of relative avidity of antibodies reactive with *Porphyromonas (Bacteroides) gingivalis* in the sera of subjects having adult periodontitis

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Relative avidities of antibodies to *Porphyromonas (Bacteroides) gingivalis* in the sera of 15 patients having adult periodontitis and 15 healthy subjects were evaluated using an ammonium thiocyanate-dissociated ELISA. Graded concentrations of ammonium thiocyanate were added to a single dilution of serum in order to dissociate low avidity antibody binding to *P. gingivalis*. The concentration of thiocyanate resulting in 50% reduction in binding (absorbance) was termed the ID_{50} for that serum. When IgG-class antibodies were examined, the ID_{50} of anti-*P. gingivalis* antibodies in the sera of patients was significantly elevated (0.96M vs 0.71M; $p<0.01$, Student's t-test). In contrast, when IgM-class antibodies were examined no significant differences in ID_{50} between patients and controls were found for *P. gingivalis* (0.54M vs 0.53M). While the ID_{50} values of patient antibodies were found to be elevated relative to those of healthy controls, comparison with antibodies from rabbits immunized with *P. gingivalis* and with ID_{50} values from other human studies suggests that adult humans, in general, produce very low-avidity antibodies to *P. gingivalis*. It is suggested that the presence of low-avidity antibodies contributes to the pathology associated with periodontal disease.

Key words: avidity – affinity – periodontitis – *P. gingivalis*

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Introduction

There have been numerous reports documenting the presence of antibodies to periodontal disease-associated microorganisms in the sera of both healthy subjects and those having periodontitis and gingivitis (reviewed in 1, 2). These reports generally describe elevated antibody titers to these microorganisms in periodontally-diseased individuals, but the actual significance of their elevation is unclear, since the antibodies may be protective, mediate adjunctive tissue destruction, or merely reflect the elevated levels of these microorganisms in the gingival sulcus. The role of avidity has rarely been examined when studying the antibodies associated with periodontal disease. Avidity is the net binding strength of the interaction between multivalent antigens and antibodies. (Affinity, in contrast, is the innate intrinsic strength of the interaction between a single antigen-binding site and a monovalent epitope). Knowledge of the avidity of an antibody-antigen interaction may be more significant to our understanding of a disease process than is the knowledge of the quantity of the antibody, because avidity more closely reflects the biological importance of that antibody. A variety of host defense activities, including complement activation (3, 4), immune elimination (5) and virus neutralization (6), are affected by the avidity of the participating antibody. Since all of these mechanisms may be participating in host reactions to periodontal disease we felt that understanding the avidity of the antibodies reactive with periodontal disease-associated microorganisms would be important. Since the antigens involved are large, complex multivalent antigens (bacterial cell wall components), which cannot be studied by classical measurements using equilibrium dialysis, an ELISA-based immune complex-dissociating assay employing the chaotropic ion thiocyanate was used

to estimate the avidity of IgG and IgM anti-*P. gingivalis* antibodies. Our original hypothesis was that when serum antibodies to *P. gingivalis* from healthy control subjects and periodontitis patients were compared patients would be expected to possess antibodies of higher avidity.

Material and Methods

Antigens

Porphyromonas gingivalis (*P. gingivalis*, ATCC 33277) was grown under anaerobic conditions (85% N_2, 10% H_2 and 5% CO_2) at 37°C in 500 ml batches as previously described (7). The cells were harvested by centrifugation at 13 000 × *g* for 20 minutes, and washed three times with phosphate-buffered saline (PBS; pH 7.4). The organisms were killed and fixed by incubation with 0.5% formaldehyde in PBS at room temperature for 18 hours on a rotary shaker. The formalized bacteria were then washed three times with PBS and stored at 4°C in the same buffer. Ovalbumin was obtained from Sigma Chemical Company (St. Louis, MO).

Rabbit antisera

Prior to immunization, blood samples (5 ml) were obtained from the marginal ear vein of each 4 New Zealand White rabbits. One millimeter of aluminum hydroxide gel adjuvant containing 1 mg of antigen was injected subcutaneously into four sites (0.25 ml/site) in the back of the rabbit. Two rabbits were injected with *P. gingivalis* and 2 with ovalbumin. Additional bleedings were taken at weekly intervals following the primary immunization. A second booster immunization was given at 4 wk post-primary immunization. All blood collected was allowed to clot a room temperature for 1 h, held at 4°C overnight and then centrifuged at 13 000 × *g* for 30 min to express the serum. Sera were aliquoted and stored at −20°C until used.

Patient and control subject sera

Sera were obtained from 15 patients (ages 23–51; 8 males and 7 females) prior to their participation in an unrelated clinical study of periodontal therapy. All patients were diagnosed as having adult periodontitis, having a mean pocket depth of 3.82 ± 0.94 mm. Controls (age- and sex-matched to patients), recruited from the university community, had no prior history of periodontal disease and had a mean pocket depth of 2.37 ± 0.22 mm. Table 1 summarizes the clinical status of the subjects.

ELISA

Antibodies in the sera of immunized rabbits reactive with either *P. gingivalis* or ovalbumin were detected with the enzyme-linked immunosorbant assay (ELISA). Formalized *P. gingivalis* cells (5 μg/ml wet weight) or ovalbumin (1 μg/ml) diluted in sodium carbonate coating buffer (pH 9.6, containing 0.02% NaN_3) were added (0.1 ml) to wells of flat-bottom microtiter plates (Immulon II™, Dynatek Laboratories, Inc., Alexandria, VA) and incubated at 37°C for 1 h. The plates were then washed with PBS containing Tween 20™ detergent (PBS-T) to remove unbound antigen. Rabbit antisera, diluted in PBS-T, were then added to the microtiter wells and incubated for 1 h at 37°C. After washing, 0.1 ml goat anti-rabbit IgG (Fc-specific; Organon Teknika Corp., West Chester, PA) diluted 1:1000 in PBS-T was added to each well and incubated for 1 h at 37°C. After washing, 0.1 ml of alkaline phosphate-conjugated rabbit anti-goat IgG (Bio-Rad Laboratories, Richmond, CA) diluted 1:500 was added to each well and incubated for 1 h at 37°C. The plates were then washed three times with PBS-T and once with substrate buffer. Sigma 104 substrate (1 mg/ml, Sigma Chemical C., St. Louis, MO) was added to each well (0.1 ml/well) and allowed to develop at room temperature for 45 min. The absorbance at 405 nm was read in a Perkin-Elmer spectrophotometer. Antibody activity was expressed as ELISA units (EU) which were calculated by a linear regression analysis of the reference serum (week 1 bleeding). EU were calculated by relating absorbance values from each sample to the reference serum, which was assigned a value of 100 EU. Only absorbance values occurring in the linear portion of the titration curves were used in these calculations.

Antibodies of the IgG and IgM classes reactive with *P. gingivalis* in the human sera were assessed in a similar fashion with the following modifications. A reference human sera pool having antibodies reactive with *P. gingivalis* was diluted and added to each plate to serve as the reference for EU calculations. Rabbit anti-human IgG or IgM (heavy chain-specific; Bio-Rad Laboratories, Richmond, CA) and alkaline phosphatase-conjugated goat anti-rabbit IgG were substituted for their counterparts in the assay described above.

Table 1. Clinical status of study participants

Category	Age (Mean ± SEM)	No. of Teeth (Mean ± SEM)	Pocket Depth (Mean ± SEM)
Controls	34.2 ± 2.3 (21–46)	26.4 ± 3.1 (23–32)	2.37 ± 0.22 (2.3–3.4 mm)
Patients	37.4 ± 3.5 (23–51)	22.8 ± 6.2 (20–32)	3.82 ± 0.94 (3.1–5.6 mm)

Dissociation analysis

Estimation of avidity was performed by measuring the dissociation of antibody-antigen binding by increasing concentrations of ammonium thiocyanate (0 to 8M) in PBS-T (8, 9). The microtiter plates were sensitized with antigen as described above. After washing with PBS-T, a single dilution of each sera was added to each well in a row in the plate. The serum dilution was chosen from the linear portion of the titration curve of that serum. After incubation at room temperature for 30 min, the plates were washed with PBS-T and increasing concentrations (0–8M; 1.0 ml/well) of ammonium thiocyanate were added to each well in a row. After an incubation for 1 h and three washes with PBS-T, the assay proceeded as described above. Percent antibody binding ([Absorbance of thiocyanate-treated well/absorbance of control well] × 100%) was calculated for each concentration of thiocyanate ion used. The concentration of thiocyanate ion required to inhibit 50% of the bound antibody (ID_{50}) was calculated by linear regression analysis of the plot of the probit values of *percent antibody binding* vs log of the *thiocyanate concentration*.

Statistical analysis

Student's t-test was used to evaluate the significance of differences in the antibody titers andID_{50} values when patient and control group sera were compared. The relationships between antibody titer and ID_{50} in these groups were tested using regression analysis.

Results

Rabbit studies

Preliminary studies designed to characterize and optimize the methodology to rank avidity were performed on bleedings obtained weekly from rabbits immunized with either ovalbumin or *P. gingivalis*.

Reversibility of ammonium thiocyanate treatment on antigens. The ranking of antibody avidity by ammonium thiocyanate dissociation of antibody-antigen complexes was possible only if the dissociating agent had no deleterious effect on the antigen, such as removing it from the plate or changing its tertiary structure, at the concentrations in which it was employed. In order to test this, microtiter wells sensitized with either *P. gingivalis* (5 μg/ml) or ovalbumin (1 μg/ml) were incubated for 1 h at room temperature with increasing concentrations of ammonium thiocyanate (0–8M). Following a washing step, antisera obtained from the wk-8 bleedings were added to the wells. The ELISA was then completed as described under Methods. The absorbances (405 nm) of the thiocyanate-treated wells, after substrate development, were compared to the control wells. As shown in Fig. 1, no significant effect on color development was noted at any thiocyanate concentration, and thus the thiocyanate did not alter either the amount or nature of the plate antigen.

Effect of thiocyanate incubation temperature. Temperature effects on dissociation by thiocyanate was next assessed by performing a 1-h thiocyanate incubation at 4°C, 27°C and 37°C. The microtiter wells were sensitized as previously described and then incubated with the wk-8 rabbit bleeding. After washing, dissociation with thiocyanate was performed for 1 h at the three temperatures described. The results are shown in Fig. 2. The effect of temperature is more noticeable when examining the anti-*P. gingivalis* antibody. Increasing the temperature creates a steeper slope in the titration curve of both antibodies (i.e., there was greater dissociation with increasing temperature).

Effect of thiocyanate incubation time. The effect of thiocyanate incubation time was assessed by comparing the 15-min, 60-min and 18-h incubations at 27°C. As shown in Fig. 3, there was no significant enhancement of dissociation as the incubation time was increased from 15 min to 18 h.

Effect of antisera dilution used in the ranking of avidity. The effect of antisera dilution on dissociation analysis was examined (10). Since the anti-ovalbumin antibody appeared to be more sensitive to treatment effects than was the anti-*P. gin-*

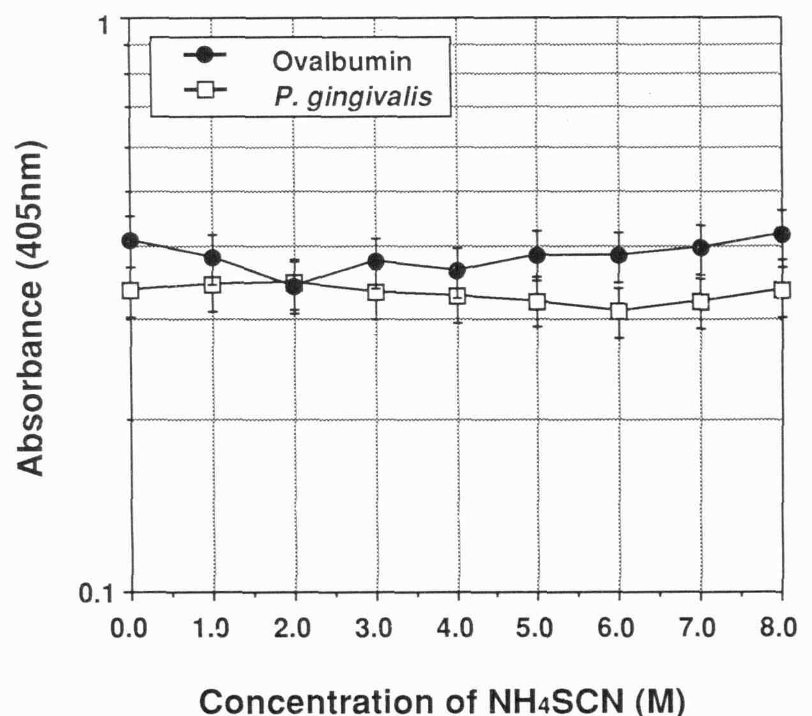

Fig. 1. Reversibility of ammonium thiocyanate treatment. Graded concentrations of thiocyanate were added to antigen-sensitized wells. After washing, anti-*P. gingivals* or anti-ovalbumin was added (1:1000 dilution of wk-8 bleeding) and then the plates were processed normally as described in Methods.

givalis, the effect of dilution was assessed in the anti-ovalbumin antisera. Dilutions were examined representing three aspects of the linear portion of the titration curve: 1/1000 (near the upper plateau); 1/4000 (middle of the curve); and 1/8000 (near the base of the curve). Dissociation analysis was performed on each dilution. The substrate development was allowed to continue until the absorbance (405 nm) reached a value of 1.0. No significant differences were found in the plots of the inhibition curves or the calculated ID_{50} values for the range of serum dilutions used. However, in order to maintain consistency in all subsequent titrations, a dilution near the upper plateau was employed in all subsequent studies.

Kinetics of avidity and titer following immunization. The ability to distinguish changes in avidity and titer were demonstrated by using the ELISA to monitor the changes in avidity and titer that occurred following immunization with the two antigens. Two representative series are shown in Fig. 4. Immunization with either *P. gingivalis* (upper panel) or ovalbumin (lower panel) results in the production of increasing titers and avidities with time. In these examples, the avidity and titer curves has distinct profiles. The 1st-wk post-immunization bleedings are used as reference sera for the EU calculations (100 EU).

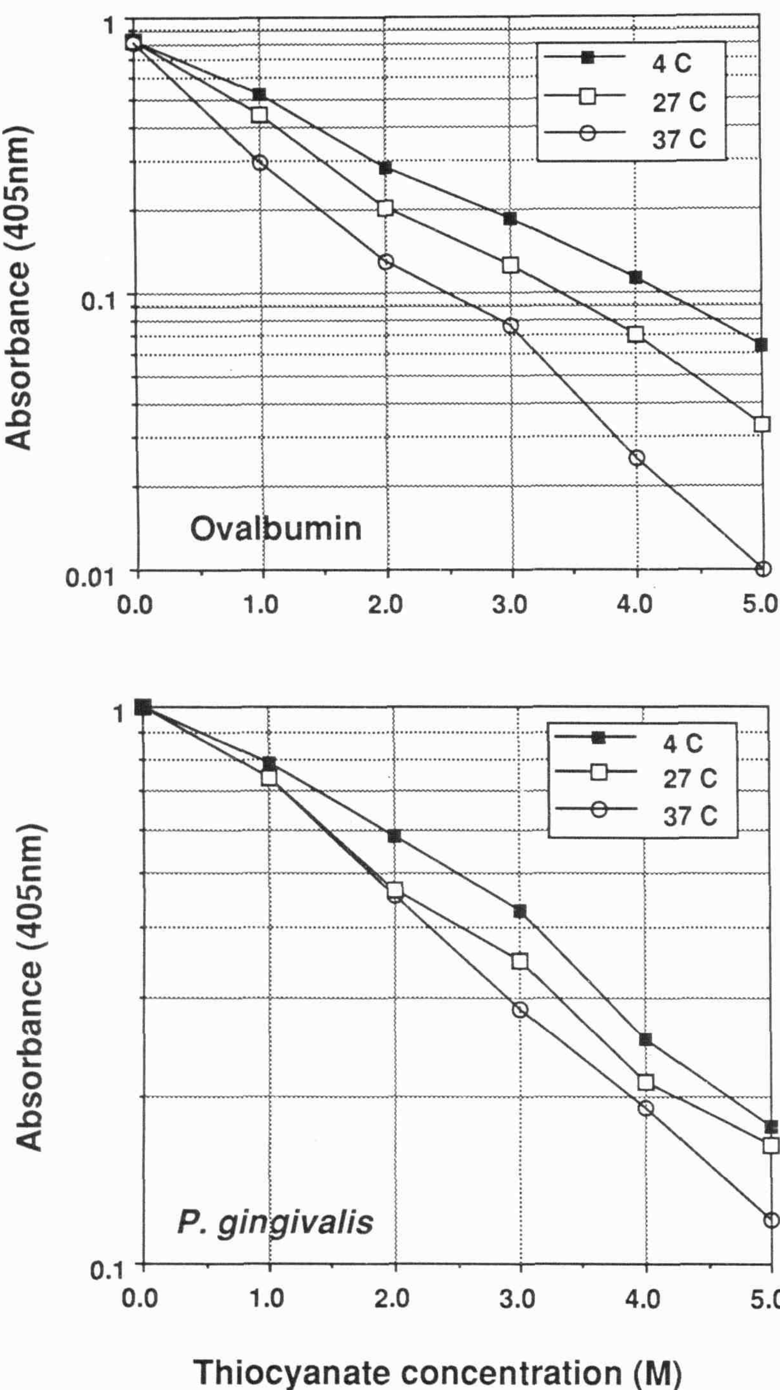

Fig. 2. Effect of temperature on thiocyanate dissociation. Thiocyanate dissociation treatment step was performed by treating wells with graded concentrations of thiocyanate and then the plates were incubated at either 4°C, 27°C or 37°C. After incubation, the plates were processed normally at 27°C. (Upper panel: Anti-ovalbumin antibody; Lower panel: Anti-*P. gingivalis* antibody).

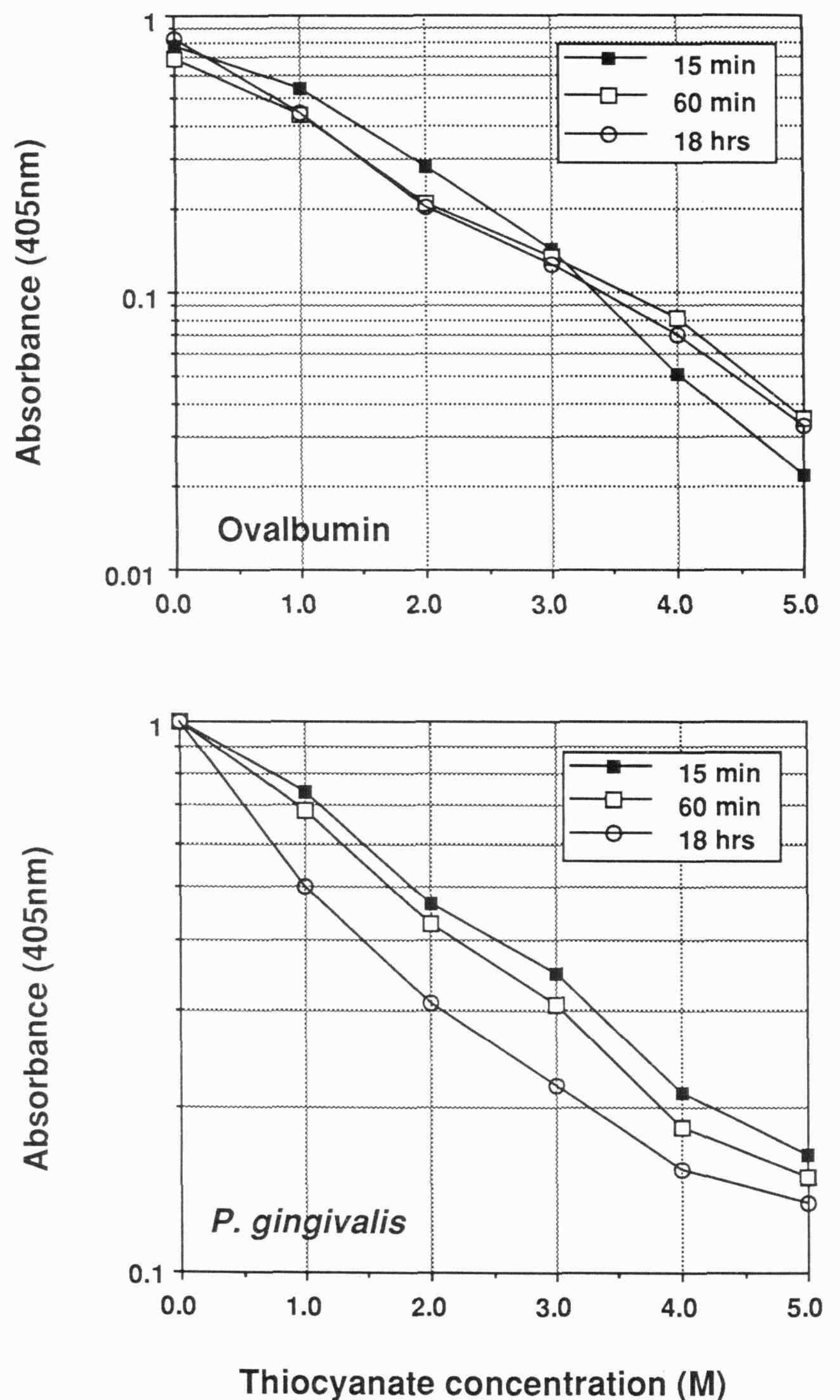

Fig. 3. Effect of incubation time with thiocyanate on dissociation. Thiocyanate dissociation treatment step was performed by treating wells with graded concentrations of thiocyanate and then the plates were incubated for 15 min, 1 h or 18 h at 27°C. After incubation, the plates were processed normally at 27°C. (Upper panel: Anti-ovalbumin antibody; Lower panel: Anti-*P. gingivalis* antibody).

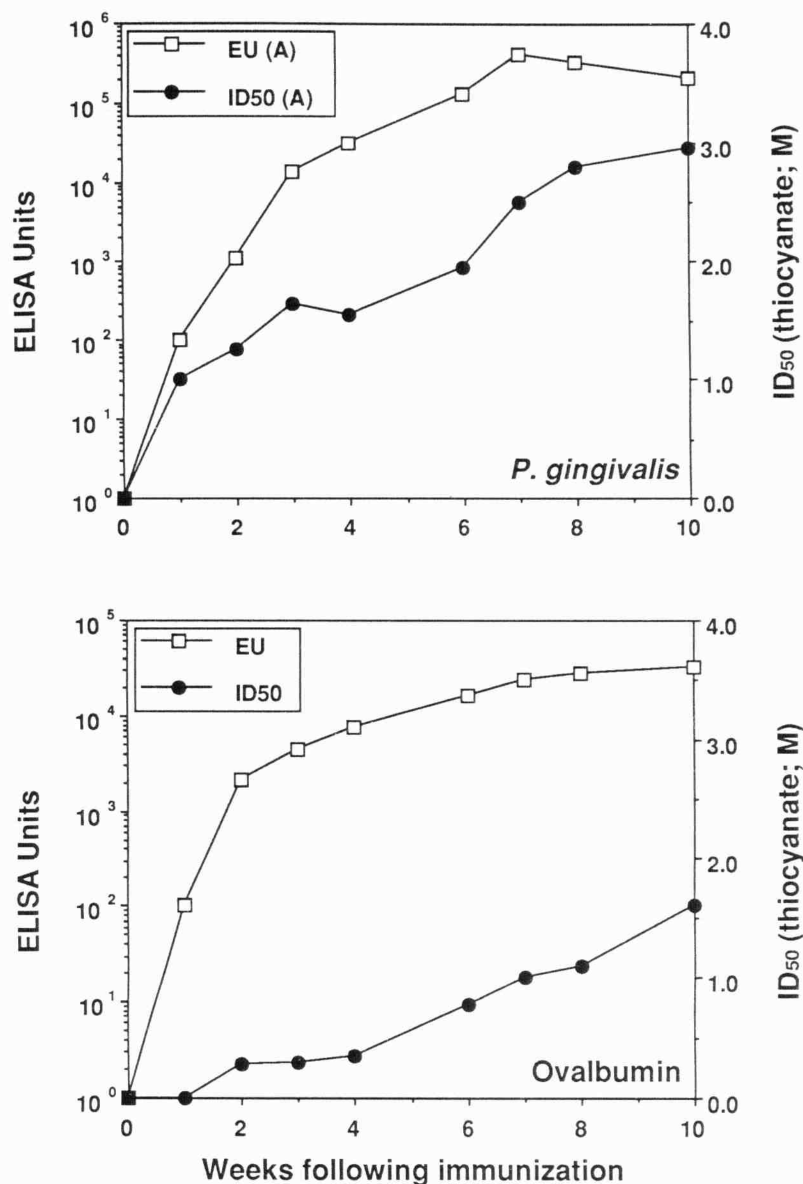

Fig. 4. Kinetics of avidity and titer following immunization. Following immunization of rabbits with *P. gingivalis* (upper panel) or ovalbumin (lower panel) bleedings were obtained at regular intervals. The relationship between titer (EU) and avidity (ID_{50}) are shown.

Human studies

Fifteen serum samples from healthy control subjects were compared to an additional 15 serum samples from subjects having chronic adult periodontitis.

Serum antibody titers. The antibody titers to *P. gingivalis* (Table 2). The mean titers of the patient sera (IgG) are significantly elevated when compared to the control sera (306.7 ± 37 EU vs. 150.6 ± 17, $p < 0.01$). No significant differences were found in the IgM class antibodies (73.8 ± 8 vs. 62.3 ± 4.0).

Avidity of patient and control sera. The avidity of anti-*P. gingivalis* antibodies of the IgG and IgM classes in the sera of adult periodontitis patients and control subjects were evaluated. When the IgG class was examined (Table 2), thiocyanate ID_{50} values of anti-*P. gingivalis* antibodies were significantly elevated (0.997 ± 0.05 vs. 0.727 ± 0.11; $p < 0.01$). In contrast, when the IgM class was examined, there was no significant difference between the two groups (0.54 ± 0.04 vs. 0.53 ± 0.04).

Relationship between titer and avidity. A regression analysis was performed to determine if there was a significant relationship between antibody titer avidity. No significant relationships existed between titer and avidity when antibodies of the IgG class were evaluated ($r^2 = 0.03$, patients; $r^2 = 0.012$, controls). This was also observed when antibodies of the IgM class were evaluated ($r^2 = 0.305$, patients; $r^2 = 0.326$, controls, Fig. 5).

Discussion

While antibody avidity and immune maturation have long been known to be important characteristics of the immune response to foreign substances, these factors have rarely been considered in the evaluation of immunity to etiologic agents or in the comparison of disease-susceptible to disease-resistant populations. Typically, the immune responses to an etiologic agent are expressed in terms of antibody titers or ELISA units without attention to the quality of the antibody in terms of avidity. However, it is clear that a variety of immune functions, including complement activation, opsonization and toxin neutralization are more effective in the presence of high-avidity antibodies (11). The humoral immune response to *P. gingivalis*, a microorganism implicated in adult periodontitis, has been examined in numerous clinical studies of both natural history (12–16) and treatment effects (17–19). In most of these studies, *P. gingivalis*-reactive antibody titers were found to be elevated in patients having common, adult-onset periodontal disease when compared to healthy age-matched control subjects. However, there have been few, if any, reports on the avidity of these antibodies. In our study, we have attempted to assess the avidity of these antibodies and to relate them to the anti-

Table 2. Antibody titer and avidity of control subject and patient serum antibodies reactive with *P. gingivalis*

	IgG Antibodies		IgM Antibodies	
Subjects	EU ± SEM	ID_{50} ± SEM	EU ± SEM	ID_{50} ± SEM
Controls	150.6 ± 17	0.727 ± 0.11	62.3 ± 4.0	0.53 ± 0.04
Patients	306.7 ± 37	0.997 ± 0.05	73.8 ± 6.6	0.54 ± 0.04
Significance* (P vs. C)	p < 0.01	p < 0.01	N.S.	N.S.

* Student t-test.

body titer and disease status of the subjects.

The complex nature and high molecular weights of the antigens involved precludes the classical method of affinity measurement, equilibrium dialysis, which employs diffisuble low molecular weight dialyzable ligands. Avidity assessments in complex antigenic systems such as rubella (20, 21), *E. coli* (22), toxoplasma (23) and tetanus (24) have relied on the use of immune complex-dissociating agents. Dissociation or denaturation analysis is based on the premise that the antigen-antibody interactions mediated by high-avidity antibodies are more difficult to disrupt than those mediated by lower avidity antibodies. Urea and the chaotropic ion thiocyanate have been used most commonly for this purpose. The avidities of antibodies can, thus, be characterized or ranked by the concentration of chaotropic agent required to disrupt them, i.e. an ID_{50}. Since these agents may also have deleterious effects on the antigen systems utilized, we first examined the effects of thiocyanate ion directly on the *P. gingivalis* antigen preparations and on *P. gingivalis*/antibody immune complexes using antibodies obtained from rabbits immunized with *P. gingivalis*. By using rabbit antibodies obtained at different stages in the immune response to *P. gingivalis* (following active immunization), representing a range of antibody affinities from very low (early primary) to very high (late secondary) we also had a reference against which we could compare the dissociation of similar human antibodies.

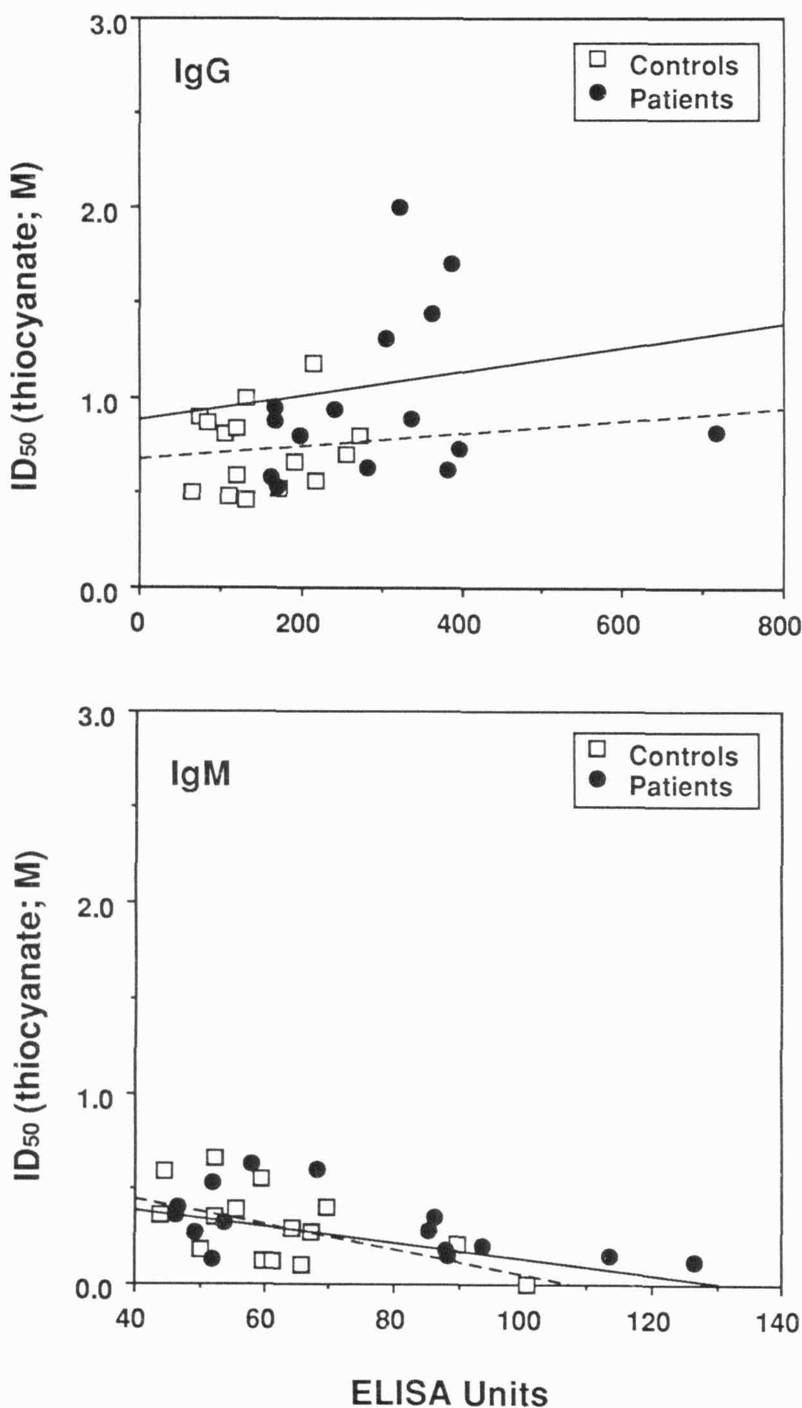

Fig. 5. Relationship between EU and avidity. The titer (EU) and avidity (ED_{50}) of antibodies reactive with *P. gingivalis* are shown for healthy subjects and patients having adult periodontitis. The best fit regression lines are indicated (solid line, patients; dashed line, controls). Antibodies of the IgG class are shown in the upper panel ($r^2=0.03$, patients; $r^2=0.012$, controls) and antibodies of the IgM class are shown in the lower panel ($r^2=0.305$, patients; $r^2=0.326$, controls).

In the preliminary studies employing rabbit antibodies, the *P. gingivalis* antigen preparations and ovalbumin were found not to be removed from the plate and to be resistant to the denaturing effects of thiocyanate. This is important since any perceived reduction in antibody titer could be interpreted as a dissociation of weak antibody-antigen complexes when in reality it was due to denaturation of the antigen or the stripping of the antigen from the microtiter plate. In this case, thiocyanate was found suitable for use in assessing the avidity of antibodies reactive with *P. gingivalis* and ovalbumin. Antibodies in bleedings obtained from rabbits 1 wk post-immunization with *P. gingivalis* possessed ID_{50} values of approximately 1M. These values rose progressively to approximately 3M by 5 wk following the booster immunization (approx. 10 wk post-primary immunization). As expected, antibody titers to *P. gingivalis* also demonstrated a progressive increase with time, but were not directly correlated to the changes in avidity. Avidity of antibodies reactive with ovalbumin, a protein with more restricted complexity and size (mol. wt. of 40 000), were not measurable until 2 wk post-immunization, but reached approximately 1.5M at 5 wk following the booster immunzation. The antibody response to ovalbumin demonstrated a progressive and significant increase in titer over time that was not directly tied to changes in avidity.

The antibody titer and avidity of anti-*P. gingivalis* antibodies of both the IgG and IgM classes in the sera of healthy subjects and periodontitis patients were assessed. In confirmation of reports by other investigators, we found the mean titer (EU) of antibodies of the IgG class to be significantly elevated in the sera of patients having adult periodontitis (306.7±37 EU vs. 150.6±17 EU). No difference was found in the titer of IgM antibodies (73.8±6.6 EU vs. 62.3±4.0 EU). Evaluation of the avidities of the IgG antibodies demon-

strated a significant elevation in the patients' sera (0.998 ± 0.11M vs. 0.724 ± 0.06, $p < 0.01$), while no difference existed in the IgM antibodies (0.54 ± 0.04 vs. 0.53 ± 0.04, N.S.). The elevated avidity of the patient IgG antibodies was not unexpected since it probably represented a more vigorous, repeated antigenic challenge with *P. gingivalis* in those subjects compared to the healthy controls. It is generally observed that acute disease or vaccination exposure to antigen leads to increased antibody titer and avidity (11). It was not unexpected to find no differences in the IgM antibody avidity since IgM antibodies are not believed to demonstrate affinity maturation. As displayed in Fig. 5, there was no direct relationship between the avidity and titer in the IgG class. This parallels the rabbit model.

While a significant increase in avidity of the IgG antibodies in the patient sera was observed ($p < 0.01$), the absolute levels of avidity were extremely low when compared to the avidity levels obtained in the rabbits immunized with *P. gingivalis* (Fig. 4). The human response to *P. gingivalis*, regardless of the periodontal disease status (health or disease), appeared comparable to the early primary response of the rabbits, which represented antibodies of quite low avidity. These preliminary observations suggest that little immune maturation of human anti-*P. gingivalis* antibodies has occurred. However, one must observe caution when comparing immune responses in different systems since immune maturation and genetic differences in responsiveness to specific antigens may exist.

It is possible to compare our results with other reports in human systems which employed the thiocyanate dissociation method to assess avidity. In studies reported by Fitzgerald *et al.* (20), thiocyanate dissociation was used to characterize the antibodies (IgG) to rubella antigen in patients after infection *in utero*. In this study, adult subjects who were exposed to rubella *in utero* demonstrated avidity values of 1.09M compared to a control group of rubella antibody-positive subjects who possessed a value of 2.57M. In that study, a value of 1.09M was considered to reflect quite low-avidity antibody. In an IgA system, the avidity of antibodies to *E. coli* and diphtheria toxin were compared in the breast milk from Swedish and Pakistani mothers (22). Differences in antigen exposure, nutritional status or genetic factors were suggested for depressed avidity in the Pakistani mothers (*E. coli*, 1.78M vs. 2.65M; diphtheria toxin, 2.35M vs. 4.30M). In comparison to these values, the value of 0.998M measured in our patients to *P. gingivalis* appears also to reflect low-avidity antibody. Comparisons with other Ig classes must be done with care since the s-IgA system represents the interaction of four antigen binding sites compared to two in the case of IgG. However, given this perspective, the statistically significant elevation of the patient avidity compared to the controls appears inconsequential when the absolute magnitudes of the values are considered. The important observation is that *both* patients and healthy subjects appear to possess low-avidity antibodies to *P. gingivalis*. Ongoing experiments in our laboratory, using the human sera from this study (unpublished), are examining the avidity of antibodies reactive with tetanus toxoid and streptokinase. Preliminary findings have revealed that the avidity of these antibodies is consistent with the previously mentioned reports (tetanus toxoid, 2.80M [controls] vs. 2.78M [patients]; streptokinase, 2.08M vs. 2.29M).

The occurrence of low avidity antibodies has been shown to be a function of a variety of factors. By far the simplest explanation, and least tenable, for the presence of low avidity antibodies might be that *P. gingivalis* present in the periodontal pockets, and other putative pockets, and other putative cross-reactive microorganisms in the host, serve as an immunoadsorbant or antibody-sponge (25) removing the higher avidity antibodies in the circulation. However, it is unlikely that this is occurring since we found the avidities of the antibodies in the control subjects to be lower than those found in the patients, who would be expected to have greater absorptive potential. In addition, since the plaque flora is generally confined to the pocket, it is more likely that antibodies in the crevicular fluid, rather than in the circulation would be removed. Other explanations might involve high antigenic doses (26), chronic exposure to antigen (27), or *in utero* antigen exposure (20) which have been shown to lead to the production of low-affinity antibody. Bacteremias associated with plaque microorganisms (28, 29) that might be more common with the often severe gingivitis associated with pregnancy (30) may result in *in utero* exposure of antigen to the developing fetus. Early chronic exposure to low level concentrations of the antigen, either *in utero* or early in life might compromise the ability to mount a significant immune response to these microorganisms by depleting the high-affinity antibody-producing clones, leaving only low-avidity capability (31), or by activating suppressor activity. Mouton *et al.* (32) have shown that immunologic exposure to periodontal disease-associated microorganisms occurs at an early age. Recent unreported studies evaluating avidity of antibodies reacting with oral microorganisms in the sera of young children ages 9–14 yr (personal communication, Dr. Michael Cole) indicate that children produce high-avidity antibodies compared to adults, thus supporting the concept that chronic exposure after

birth, rather than *in utero* may be responsible for the low avidity of the adult antibodies. Since our present study lacks information on subgingival plaque flora and duration of antigenic exposure, factors which are important in the affinity maturation process, it is difficult to address the actual mechanism(s) involved in our observations.

It is clear from earlier reports that protection against disease is a function of antibody avidity to the etiology agent (33). It is generally advantageous to develop high-avidity antibody to an antigen. This is supported by a range of biological mechanisms that are more effective with high avidity, including: toxin neutralization (34), destruction of D+ erythrocytes (35), passive hemagglutination (36), complement fixation (4, 37), passive cutaneous anaphylaxis (4, 37), hemolysis (37), immune elimination of antigen (5), virus neutralization (6), damage to DNP-sensitized liposomes (3), inactivation of enzymes (38), protection against bacterial infections (39), blocking antibodies (26), and anti-GMB antibody binding (40). These studies suggest that an affinity (K_0) difference of only 10-fold significantly compromises the efficiency of the biological reaction. In addition, experimental data in animals demonstrate more severe and chronic disease in the presence of low-avidity antibodies, attributable to a greater difficulty in eliminating antigen and more tissue localization of antigen-excess complexes (5).

The observation that human antibodies to *P. gingivalis* in general appear to be of low avidity presents a new insight into the destructive pathology associated with adult periodontitis and with the role of the elevated antibody titers to the periodontal disease-associated microorganisms. These data suggest that either affinity maturation or the ability to express high-avidity antibodies reactive with these microorganisms might be compromised. We theorize that such compromise may occur as a result of *in utero* tolerization to bacterial antigens or, more likely, as a result of chronic exposure to the developing oral flora in the neonatal and preadult period. However, while the mechanism remains to be elucidated, the presence of elevated antibody titers at best reflects the presence of increased levels of the microorganisms in the pocket. The effectiveness of the antibody in clearance and elimination mechanisms would be expected to be poor and the innate destructive nature of the bacterial flora would be uncompromised. While there is a significant elevation of antibody avidity to *P. gingivalis* in the sera of patients relative to that of controls, the absolute levels of avidity compared to other antigen systems is extremely depressed. This observation is currently being confirmed with additional antigens and human sera.

Acknowlegment

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