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Immunoglobulin G Subclass Response of Localized Juvenile Periodontitis Patients to *Actinobacillus actinomycetemcomitans* Y4 Lipopolysaccharide

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Sera from patients with localized juvenile periodontitis (LJP) often contain markedly elevated immunoglobulin G (IgG) antibody titers to serospecific determinants of the lipopolysaccharide (LPS) from *Actinobacillus actinomycetemcomitans*. The objective of the present study was to define the subclass distribution of the IgG antibody response of LJP patients to this key cell envelope antigen. IgG subclass antibody responses to *A. actinomycetemcomitans* LPS were quantified in an enzyme-linked immunosorbent assay with human IgG subclass-restricted monoclonal antibodies. Serum antibody concentrations were calculated by heterologous interpolation of a dose-response curve constructed by using human-mouse chimeric antibodies. Sixteen of 17 LJP serum samples tested contained significantly greater concentrations of IgG2 than IgG1 antibodies reactive toward *A. actinomycetemcomitans* LPS. Geometric mean antibody concentrations of IgG1 and IgG2 were 7.8 and 136.5 μg/ml, respectively, among LJP patients with elevated IgG titers to LPS (94% of whom were black). However, both IgG1 and IgG2 antibody concentrations were significantly greater than the corresponding values obtained from sera from LJP patients with low IgG titers to LPS. Among LJP patients with elevated IgG titers to *A. actinomycetemcomitans* LPS, serum IgG2 concentration and total IgG concentration were also significantly elevated compared with both low-titered LJP sera and sera from periodontally healthy race-matched controls. The results of this study indicate that the humoral response of a predominantly black population of LJP patients to *A. actinomycetemcomitans* includes the production of LPS-reactive IgG antibodies which are primarily of the IgG2 subclass.

Actinobacillus actinomycetemcomitans is a gram-negative, capnophilic, fermentative coccobacillus which is frequently isolated from lesions of patients with localized juvenile periodontitis (LJP) (24, 33). LJP patients colonized by *A. actinomycetemcomitans* often exhibit markedly elevated titers of serum, salivary, and crevicular fluid immunoglobulin G (IgG), IgA, and IgM antibody against this organism (4, 6, 7) compared with those in sera from periodontally healthy persons. LJP sera have been shown to contain IgG antibodies reactive toward leukotoxin (28), outer membrane proteins (30, 31), and lipopolysaccharide (LPS) (8, 32). Nevertheless, detailed information regarding the functional capabilities of IgG antibody against somatic antigens of *A. actinomycetemcomitans* is presently lacking.

It has been recognized for some years that human serum IgG is composed of four distinct subclasses (12, 25). The IgG subclasses differ not only in their distribution in normal serum, but also in their biologic properties and the nature of the antigens which elicit their production (10). One important biologic function of IgG involves activation of the complement cascade, which can initiate a number of protective reactions including phagocyte chemotaxis, membranolytic attack on susceptible gram-negative organisms, immune adherence, and opsonization. Whereas IgG1 and IgG3 are potent complement activators, IgG2 is comparatively weak in this regard. IgG4 appears to be inactive in fixing complement via the classical pathway. A second important biologic function of IgG involves binding to membrane Fc receptors, particularly on phagocytic cells. Both mononuclear and polymorphonuclear phagocyte Fc receptors exhibit preferential binding of IgG1 and IgG3 over IgG2 and IgG4 (2, 10). Given differences in the functional properties of the four IgG subclasses, the predominant IgG subclass response elicited during natural infection or vaccination may influence the capacity of the humoral response to provide adequate host defense.

Analysis of the IgG subclass pattern of antibodies produced after natural infection or vaccination indicates that protein and polysaccharide antigens evoke distinct IgG subclass responses (16, 17). Bacterial protein antigens preferentially induce IgG1 antibodies in humans, with minor contributions of IgG3 and IgG4. In contrast, IgG responses to polysaccharides are normally restricted to the IgG2 subclass, although some polysaccharides also induce substantial amounts of IgG1, particularly in children.

The immunodominant antigen of *A. actinomycetemcomitans* Y4 was first reported to be a carbohydratelike species which contains a serospecific determinant(s) (4). We subsequently demonstrated that the polysaccharide moiety of LPS defines the serologic specificity of *A. actinomycetemcomitans* (32). Moreover, LPS was found to represent a major target for IgG antibody in sera from LJP patients colonized by this organism. In the present study, we examined the IgG subclass response of LJP patients to isolated LPS from *A. actinomycetemcomitans*. LPS-specific IgG subclass antibodies were quantified in an enzyme-linked immunosorbent assay (ELISA) with subclass-specific monoclonal antibod-

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ies. Serum antibody concentrations of each subclass were calculated by using a chimeric antibody-based calibration procedure. The results of this study indicate that sera from LJP patients contain IgG antibodies toward *A. actinomycetemcomitans* LPS which are mainly of the IgG2 subclass.

MATERIALS AND METHODS

Patient serum samples. Serum samples were obtained from 23 patients with LJP (mean age, 18.4 years; range, 10 to 31 years; 19 black, 3 Caucasian, 1 Oriental) receiving treatment through the Periodontal Disease Clinical Research Center, State University of New York at Buffalo. Diagnosis of LJP was made on the basis of alveolar bone loss and periodontal destruction limited to the first molars and incisors and not more than two additional teeth. IgG antibody titers to *A. actinomycetemcomitans* LPS were determined by ELISA as described previously (32). On the basis of these results, the sera were subdivided into two groups: (i) high-titered LJP sera (geometric mean IgG antibody titer to LPS, 36,613; n = 17), and (ii) low-titered LJP sera (geometric mean IgG antibody titer to LPS, <125; n = 6). Serum samples from eight periodontally healthy black individuals (age range, 15 to 22 years) were kindly provided by H. Schenkein, Medical College of Virginia, Richmond.

LPS isolation. LPS was obtained from thioglycolate-grown *A. actinomycetemcomitans* ATCC 43718 (strain Y4) as described previously (32). Briefly, the organisms were extracted into hot aqueous phenol. The crude phenol-water extract was chromatographed on Sephacryl S-400 in LPS-disaggregating buffer, subjected to nuclease digestion, and rechromatographed. High-molecular-weight LPS thus obtained was essentially free of contaminating nucleic acid or protein and formed a precipitin band with rabbit serotype b-specific antiserum but not with antiserum specific for serotype a or c. In addition, rabbit serotype b antiserum and LJP sera formed a line of identity with respect to this antigen.

IgG subclass ELISA. Serum IgG subclass concentrations of antibody reactive toward *A. actinomycetemcomitans* LPS were determined by a modification of an ELISA procedure described previously (32). Briefly, the top two rows of each immunoassay plate (Costar, Cambridge, Mass.) were coated with a conjugate consisting of the hapten 4-hydroxy-3-nitrophenylacetyl (NP) coupled to human serum albumin. This conjugate was prepared by coupling NP-*O*-caproic acid–succinimide ester in *N,N'*-dimethylformamide to human serum albumin in a 10:1 molar ratio. The conjugate was exhaustively dialyzed against phosphate-buffered saline (PBS) and stored at 1 mg/ml at −20°C. Each well was coated with 0.1 ml of an optimal concentration of NP-human serum albumin conjugate (5 μg/ml in PBS, pH 7.4). The remaining rows of each plate were coated with 0.1 ml per well of *A. actinomycetemcomitans* LPS (10 μg/ml) in 0.015 M carbonate (pH 9.6). The plates were incubated overnight at 4°C and washed three times in PBS-0.05% Tween 20. The plates were then incubated for 2 h in blocking buffer consisting of PBS-1% bovine serum albumin (BSA) (fraction V; Sigma Chemical Co., St. Louis, Mo.). Unbound protein was removed by washing in PBS–0.05% Tween 20. Serial twofold dilutions of patient sera were prepared in PBS-1% BSA. A 0.1-ml sample of each dilution was transferred to duplicate LPS-coated wells and incubated overnight at 23°C. The plates were washed and subsequently incubated with 0.1 ml per well of biotinylated murine monoclonal antibodies specific for human IgG1 (HP6069; Calbiochem, La Jolla, Calif.), IgG2 (HP6002), IgG3 (HP6047), or IgG4 (HP6023), each at a concentration of 1 μg/ml in PBS-1% BSA (18). The plates were incubated for 2 h at 23°C and washed three times in PBS-Tween 20. The plates were then incubated for 1 h at 23°C with 0.1 ml per well of avidin-alkaline phosphatase (Bio-Rad Laboratories, Richmond, Calif.) diluted 1:1,000 in PBS-1% BSA. The plates were washed three times in PBS-Tween 20 and once in substrate buffer (1 M diethanolamine [pH 9.8], containing 0.5 mM $MgCl_2$). The wells were then incubated at 23°C with 200 μl of *p*-nitrophenyl phosphate (Sigma) at 1 mg/ml in diethanolamine buffer. Color development was terminated by the addition of 30 μl of 1 N NaOH per well, after which the A_{405} was measured with a microplate reader (Bio-Rad model 3550). Negative controls included (i) LPS-coated wells reacted with monoclonal subclass-specific antibody and avidin-alkaline phosphatase conjugate and (ii) mock-coated wells incubated with patient sera, monoclonal antibody, and avidin-alkaline phosphatase conjugate.

To define the relationship between optical density readings and IgG subclass concentration, we constructed a heterologous dose-response curve in each immunoassay plate. The curve was generated by using a genetically engineered chimeric human-mouse antibody with mouse variable regions that define specificity for the hapten NP and human constant-region genes for IgG1, IgG2, IgG3, or IgG4 (13). Documented murine monoclonal antibodies specific for the human Ig isotypes have been used to confirm the human IgG subclass restriction of the chimeric antibodies (13). Serial twofold dilutions of each chimeric antibody were prepared in PBS-1% BSA (1,000 to 1 ng/ml), and 0.1 ml of each dilution was transferred to duplicate wells coated with NP-human serum albumin. This procedure was performed during the same time in which serially diluted patient sera were added to LPS-coated wells of each plate. In all subsequent steps, wells incubated with chimeric antibody or patient serum were treated in an identical manner. Because of the high affinity of the chimeric antibodies for NP, greater than 90% of the antibody added to each well binds to its antigen during a first incubation, as demonstrated by sequential transfer experiments (14). Accordingly, a heterologous dose-response curve is generated in which optical density can be related to the amount of chimeric antibody bound. In regions of parallelism between the chimeric antibody and serum dilution curves, the amount of IgG subclass antibody bound to LPS-coated wells can be interpolated from the chimeric antibody curve. This technique is referred to as heterologous interpolation. The use of this method in determining the concentration of IgG1 antibody to *Haemophilus influenzae* type b polyribose phosphate has recently been reported (14).

Determination of total IgG and IgG subclass concentration. Serum IgG subclass concentrations were determined by an immunoassay with subclass-specific monoclonal antibodies as described previously and utilizing WHO 67/97 serum as the reference standard (15). Total serum IgG concentrations were calculated by summation of the individual subclass concentrations. Total IgG concentration was also determined independently by radial immunodiffusion. The sum of IgG1 to IgG4 agreed to within 15% of the total IgG concentration determined independently by radial immunodiffusion (Kallestad, Austin, Tex.).

Statistical analyses. Statistical differences in LPS-specific IgG subclass antibody concentrations among LJP patients with elevated titers to LPS were determined by the Wilcoxon signed rank test. Differences in IgG antibody

TABLE 1. Levels of IgG subclass antibodies against *A. actinomycetemcomitans* Y4 LPS in LJP sera

Patient group[a]	Concn of LPS-reactive antibody (μg/ml)[b]			
	IgG1	IgG2	IgG3	IgG4
Elevated IgG titer to *A. actinomycetemcomitans* LPS (n = 17)	7.78[c] (0.3–167.1)	136.54[c,d] (3.7–1,116.8)	0.64 (0.03–27.0)	0.01 (0.01–0.03)
Low IgG titer to *A. actinomycetemcomitans* LPS (n = 6)	0.12 (0.07–0.27)	0.07 (0.02–0.39)	ND[e]	ND

[a] LJP patients were subdivided into groups with high ELISA titers of IgG antibody reactive with *A. actinomycetemcomitans* Y4 LPS (geometric mean IgG titer, 36,613) or with low IgG titers to this antigen (geometric mean IgG titer, <125), as described previously (32).
[b] Geometric mean concentration. Range of values is indicated in parentheses.
[c] Significantly different from mean IgG subclass concentration of low titered group ($P < 0.0004$) by Mann-Whitney test.
[d] Significantly different from geometric mean IgG1 subclass concentration of high-titered LJP group ($P < 0.0004$) by the Wilcoxon signed rank test.
[e] ND, not determined.

subclass concentration between patients with high and low titers were determined by the Wilcoxon-Mann-Whitney test. Differences in total and IgG subclass concentrations in sera from high-titer, low-titer, and periodontally healthy control individuals were assessed by one-way analysis of variance with pairwise comparisons, using the Fisher multiple comparison procedure. Correlation analyses were performed by using the Pearson correlation coefficient after log transformation of the data.

RESULTS

Concentration of IgG subclass antibodies to *A. actinomycetemcomitans* LPS in LJP sera. LJP sera often contain markedly elevated serum IgG antibody titers to high-molecular-weight LPS extracted from *A. actinomycetemcomitans* (32). In the present study, we examined the subclass composition of IgG antibody to *A. actinomycetemcomitans* LPS in serum samples from 17 LJP patients with elevated IgG titers to this antigen. As shown in Table 1, the geometric mean concentration of LPS-specific IgG2 antibody in high-titered LJP sera was significantly greater than that of other IgG subclasses. The mean IgG2 concentration in this group was more than 17-fold greater than the mean IgG1 antibody concentration. The IgG3 and IgG4 antibody concentrations were 1 to 2 orders of magnitude lower than the mean concentration of IgG1 antibody to *A. actinomycetemcomitans* LPS. The geometric mean antibody concentrations of IgG1 and IgG2 were significantly greater in sera from the group with high titers than in sera from six LJP patients whose IgG titers to *A. actinomycetemcomitans* LPS were minimal (reciprocal titer, <125). These results indicate that IgG antibodies in LJP sera that are reactive toward *A. actinomycetemcomitans* LPS are principally of the IgG2 subclass. However, significantly elevated levels of IgG1 antibody to this antigen are also present in sera from LJP patients who are high responders.

Among the group of 17 LJP patients whose serum IgG titers to *A. actinomycetemcomitans* LPS were elevated, 16 (94%) showed a predominant IgG2 response to this polysaccharide antigen (Fig. 1). The remaining patient had modest and approximately equal concentrations of IgG1 and IgG2 antibody to LPS (6.2 versus 3.7 μg/ml, respectively). The ratio of IgG2/IgG1 antibody was calculated for each subject in this group. The mean ratio of IgG2/IgG1 antibody to LPS was 87.2.

Relationship between IgG titer and concentration of IgG subclass antibody to *A. actinomycetemcomitans* LPS. Among LJP patients with elevated IgG titers to *A. actinomycetemcomitans* LPS, reciprocal titers showed substantial variation (range, 5,537 to 125,611). We sought to establish the relationship between IgG antibody titer to LPS and IgG subclass antibody concentration. IgG titer to LPS showed a modest correlation with IgG1 antibody concentration (Fig. 2A) and a poor correlation with IgG3 antibody concentration (Fig. 2C). No correlation analysis was performed with respect to IgG4, inasmuch as IgG4 subclass-specific antibody to LPS was virtually undetectable in most sera tested. IgG titer was found to be highly correlated (r = 0.916) with the concentration of IgG2 subclass antibody to *A. actinomycetemcomitans* LPS (Fig. 2B). These findings provide further evidence that LPS-specific IgG2 antibodies account for most of the total IgG reactive toward this antigen.

Total IgG and IgG subclass concentrations in LJP sera. We wished to determine whether the marked elevation in LPS-specific IgG2 antibodies noted in several of the high-responder LJP patients was manifest in an increase in the concentration of serum IgG2 and/or total IgG. Accordingly, total and subclass IgG concentrations were quantified in sera from patients with high or low IgG titers to *A. actinomycetemcomitans* LPS, as well as in periodontally healthy black

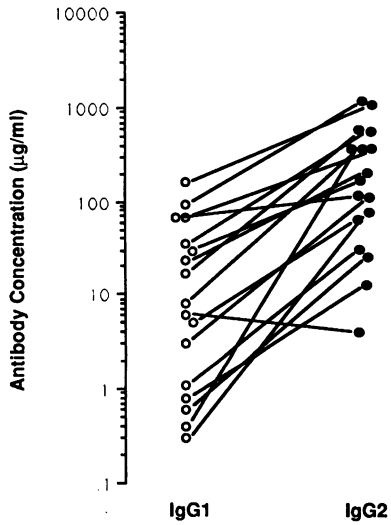
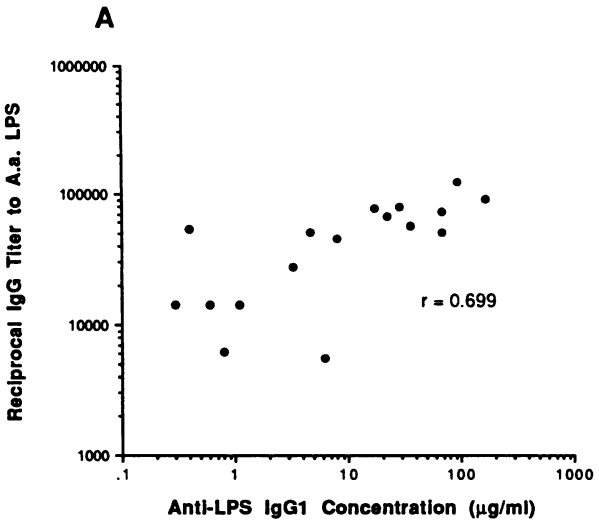
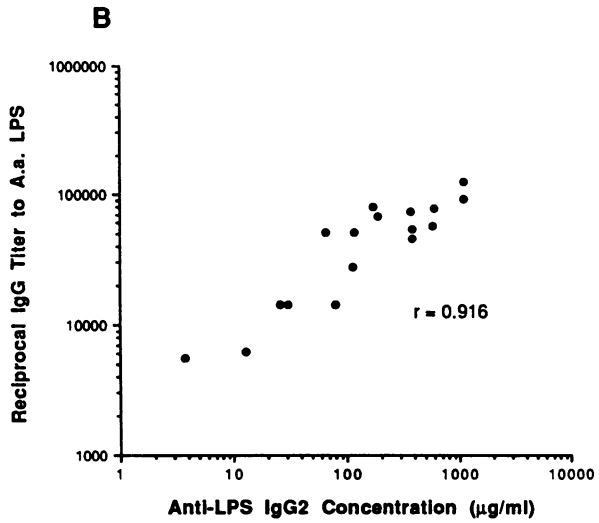


FIG. 1. Concentrations of IgG1 and IgG2 subclass antibodies to *A. actinomycetemcomitans* LPS in high-responder LJP serum samples. LPS-specific IgG subclass antibody concentrations were determined in serum samples from 17 LJP patients by an ELISA with human subclass-restricted monoclonal antibodies. Antibody concentrations were derived by heterologous interpolation of a reference curve generated by using human-mouse chimeric antibodies.





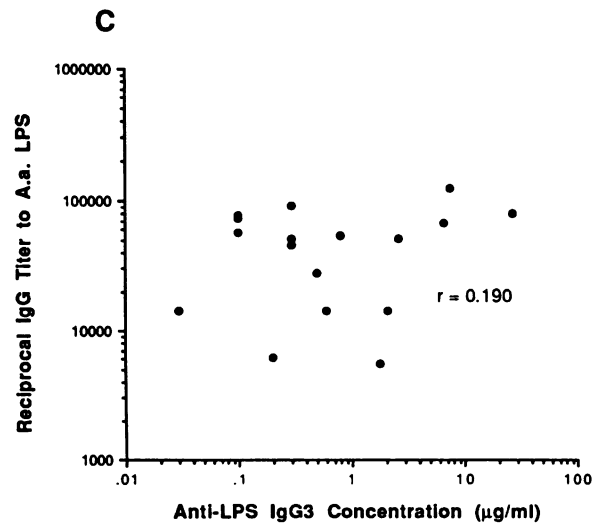


FIG. 2. Relationship between reciprocal IgG antibody titer to *A. actinomycetemcomitans* (A.a.) LPS and concentration of IgG subclass antibodies to LPS in high-responder LJP sera. Titer of IgG antibody against *A. actinomycetemcomitans* LPS was plotted as a function of antibody subclass concentration with respect to IgG1 (A), IgG2 (B), or IgG3 (C). Correlation analyses were performed by using the Pearson correlation coefficient after log transformation of the data.

individuals. As shown in Table 2, serum IgG2 concentration was significantly elevated ($P < 0.001$) in four LJP patients whose IgG titers to LPS were elevated compared with sera from low-titered LJP patients or periodontally healthy race-matched controls. The concentrations of IgG1, IgG3, and IgG4 in serum did not differ significantly between the three groups. Total IgG concentration was significantly elevated ($P < 0.01$) in the high-responder LJP patients compared with the periodontally healthy group, but was not significantly different from the mean IgG concentration in the low-titered LJP group.

Utilizing the concentration of LPS-specific IgG2 antibody and the corresponding serum IgG2 concentration, it was possible to calculate the percentage of IgG2 which was specific for *A. actinomycetemcomitans* LPS in serum samples from four high-titered LJP patients. LPS-specific IgG2 antibody varied from 1.6 to 23.4% of the total serum IgG2 in these patients. Although these results should be viewed with caution because of the limited sample size, they suggest that synthesis of IgG2 antibodies to *A. actinomycetemcomitans* LPS constitutes a major part of the overall humoral response of some LJP patients to this organism.

DISCUSSION

Among LJP patients colonized by *A. actinomycetemcomitans*, serotype b strains are recovered more frequently than serotype a or c (5, 34). This has prompted interest in defining the nature of the serotype b antigen and the host response to this cell surface component. A serotype-specific component with carbohydratelike characteristics was reported to represent an immunodominant antigen of *A. actinomycetemcomitans* Y4 (4). In a recent study, we provided evidence indicating that the polysaccharide moiety of LPS defines serologic specificity for this organism (32). Further, we observed that LJP sera often contain markedly elevated serum IgG antibody titers to *A. actinomycetemcomitans* LPS. These findings have recently been confirmed independently (20).

Polysaccharide antigens generally induce the production of IgG antibodies which are primarily of the IgG2 subclass. Such antibodies exhibit comparatively weaker complement-fixing ability and opsonic activity than antibodies of the IgG1 and IgG3 subclasses. In light of evidence indicating that LPS constitutes the immunodominant antigen of *A. actinomycetemcomitans*, we sought to characterize the IgG subclass response to this cell envelope polysaccharide. In the present study, we quantified the IgG subclass antibody response to isolated *A. actinomycetemcomitans* LPS in sera from LJP subjects with low or elevated IgG titers to this antigen. The results indicate that IgG antibodies directed toward *A. actinomycetemcomitans* LPS are primarily of the IgG2 subclass. Among patients with elevated titers to LPS, the geometric mean IgG2 antibody concentration was 17.5 times the mean IgG1 antibody concentration. The geometric mean concentration of IgG1 subclass antibody to LPS was, however, significantly greater among LJP patients with elevated titers to LPS than among patients with low titers to this antigen. Concentrations of IgG3 and IgG4 subclass antibod-

TABLE 2. Total IgG and IgG subclass concentrations in sera from LJP patients and periodontally healthy persons

Patient code	Serum conc (g/liter)[a]				
	Total IgG[b]	IgG1	IgG2	IgG3	IgG4
LJP patients with elevated IgG titers to *A. actinomycetemcomitans* Y4 LPS					
1	18.40	9.65	7.25	1.09	0.41
2	16.70	8.82	6.36	1.24	0.28
3	16.41	10.13	4.78	1.07	0.43
4	15.98	6.77	7.21	0.91	1.09
Mean ± SD	16.87 ± 1.06[c]	8.84 ± 1.48	6.40 ± 1.15[d]	1.08 ± 0.13	0.55 ± 0.36
LJP patients with low IgG titers to *A. actinomycetemcomitans* Y4 LPS					
5	11.97	7.23	3.93	0.56	0.25
6	10.60	6.74	2.86	0.57	0.43
7	9.97	6.35	1.62	1.81	0.19
8	18.52	15.07	1.67	1.27	0.51
Mean ± SD	12.76 ± 1.96	8.85 ± 4.16	2.52 ± 1.10	1.05 ± 0.60	0.34 ± 0.15
Periodontally healthy black persons					
09	10.94	5.49	4.69	0.65	0.11
10	11.48	5.63	4.21	1.12	0.44
11	13.42	9.18	3.14	0.36	0.74
12	12.31	9.86	1.66	0.71	0.08
13	9.16	5.24	2.25	0.81	0.86
14	12.80	6.61	4.15	0.39	1.65
15	6.57	4.15	1.82	0.44	0.16
16	15.26	10.33	3.32	0.96	0.65
Mean ± SD	11.49 ± 2.68	7.06 ± 2.38	3.15 ± 1.15	0.68 ± 0.28	0.59 ± 0.52
Normal range[e]	3.8–15.0	1.8–7.8	1.0–4.6	0.3–1.4	0.08–1.8

[a] Subclass concentrations were determined by enzyme immunoassay.
[b] Total IgG was calculated by summation of the IgG1 to IgG4 subclass concentrations. These summated values were within 15% of the total IgG concentration determined by an independent method (radial immunodiffusion).
[c] Significantly different from periodontally healthy group ($P < 0.01$, as determined by one-way analysis of variance by the Fisher multiple comparison procedure).
[d] Significantly different from low-titered LJP and periodontally healthy groups ($P < 0.001$, by one-way analysis of variance).
[e] Normal values (95% confidence intervals) are from Papadea et al. (21).

ies to *A. actinomycetemcomitans* LPS were quite low in both LJP groups.

The concentration of IgG2 antibody to *A. actinomycetemcomitans* LPS varied widely among high-responder LJP patients. However, the lowest concentration of IgG2 antibody detected in this group was still more than 50-fold greater than the mean IgG2 concentration among low-responder LJP patients. Ninety-four percent of the sera from high-responder LJP patients contained IgG2 antibody concentrations which exceeded their corresponding IgG1 concentration. Correlation studies defining the relationship between IgG titer to *A. actinomycetemcomitans* LPS and concentration of LPS-specific IgG subclass antibody provided further evidence that IgG antibodies to *A. actinomycetemcomitans* LPS are principally of the IgG2 subclass. Thus, IgG antibody titer was highly correlated ($r = 0.916$) with IgG2 subclass antibody concentration and moderately correlated ($r = 0.699$) with IgG1 antibody concentration.

Given the marked elevation in serum IgG2 (and IgG1) antibody concentration to *A. actinomycetemcomitans* LPS in high-responder LJP patients, we considered that the antibody response might also be reflected in an increase in the concentration of serum IgG subclasses and/or total IgG. In this context, Gunsolley and coworkers (11) noted a higher prevalence of seropositivity for *A. actinomycetemcomitans* among black LJP patients than among white LJP patients. These investigators emphasized the importance of employing race-matched controls in studies defining antibody reactivity to *A. actinomycetemcomitans* in LJP patients. Consistent with these previous observations, 16 of 17 of the high-responder LJP patients included in the present study were black. Accordingly, concentrations of total IgG and IgG subclasses in sera from our high-responder patients were compared with values derived from periodontally healthy black persons.

The concentration of IgG2 in serum was significantly elevated among four high-responder LJP patients compared with either low-responder LJP patients or periodontally healthy, race-matched controls. Mean IgG2 concentration in this group was also higher than the mean IgG2 concentration reported for a racially unspecified normal adult population (21). The mean concentration of total serum IgG was also significantly elevated in the high-responder LJP group. In contrast, concentrations of the remaining three IgG subclasses in serum did not differ among patient and control groups. It is interesting to note that the mean total IgG concentration among periodontally healthy black persons tended toward the upper end of the normal range of values. This was also the case with respect to serum IgG1 concentration. Such observations appear to lend credence to the suggestion that racial factors be considered in evaluating humoral responses of LJP patients.

The elevation in total serum IgG concentration among high-responder LJP patients is consistent with the findings of a previous study (29). In this earlier study, total IgG and IgG subclass concentrations were compared between a group of nine LJP patients (seven of whom were black) and a periodontally healthy group (race not specified). Whereas the mean concentration of IgG in serum was elevated in the LJP group, no significant differences were noted with respect to IgG subclass concentrations. These investigators noted, however, that the IgG1 and IgG2 values of the LJP patients tended toward the upper end of the normal range. In the present study, significant elevation of IgG2 concentration was detected only among LJP patients whose IgG titers to *A. actinomycetemcomitans* LPS were markedly elevated. Hence, aberrations in serum IgG subclass concentrations in LJP patients appeared to be restricted to a subset of patients characterized by a strong humoral response to *A. actinomycetemcomitans* (and, perhaps, other periodontal organisms as well).

Our finding that *A. actinomycetemcomitans* LPS stimulates production of IgG antibodies which are principally of the IgG2 subclass is consistent with other studies indicating that polysaccharide antigens in general preferentially induce IgG2 (and, to a lesser extent, IgG1) in normal adults (16, 17). A number of factors appear to influence the composition of the IgG subclass response to various antigens, including subject age, the chemical nature of the antigen, the immunologic status of the individual, and certain hereditary factors (10).

Genes associated with certain immunoglobulin allotypes, including Km(1) and G2m(n), have been reported to influence the magnitude of the IgG antibody response to bacterial polysaccharide antigens (1, 9, 10, 23). G2m(n), also referred to as G2m(23), is presently the only known allotype for the constant region of the γ2 heavy chain (27). Adult subjects who are positive for G2m(n) exhibit significantly greater IgG2 antibody responses to pneumococcal polysaccharides and *H. influenzae* type b polysaccharides than subjects who are negative for this allotype marker (1, 10, 23). Gm allotype-associated differences in the concentrations of human IgG subclasses in serum have also been observed (19, 22, 26). Notably, mean serum IgG2 concentration is highest among subjects who are positive for the G2m(n) allotype. The influence of the G2m(n) allotype in defining the magnitude of the IgG2 response to *A. actinomycetemcomitans* LPS warrants further study.

LPS has been reported to represent an immunodominant surface antigen of *A. actinomycetemcomitans*. The results of the present study indicate that IgG antibodies directed toward this cell envelope constituent are primarily of the IgG2 subclass. The production of poorly opsonizing and weakly complement-fixing IgG2 antibodies against this key surface antigen may limit antibody-mediated host defense against *A. actinomycetemcomitans*. However, LJP sera have been reported to contain opsonic IgG antibody activity against this organism (3). Whether such antibody recognizes LPS or outer membrane proteins has not been established. Current efforts are directed toward defining the IgG subclass and antigenic specificity of opsonic antibody against *A. actinomycetemcomitans*.

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REFERENCES

1. **Ambrosino, D. M., G. Schiffman, E. C. Gotschlich, P. H. Schur, G. A. Rosenberg, G. G. DeLange, E. van Loghem, and G. R. Siber.** 1985. Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. J. Clin. Invest. **75:**1935–1942.
2. **Anderson, C. L., and R. J. Looney.** 1986. Human leukocyte IgG Fc receptors. Immunol. Today **7:**264–266.
3. **Baker, P., and M. Wilson.** 1989. Opsonic IgG antibody against *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis. Oral Microbiol. Immunol. **4:**98–105.
4. **Califano, J. V., H. A. Schenkein, and J. G. Tew.** 1989. Immunodominant antigen of *Actinobacillus actinomycetemcomitans* Y4 in high-responder patients. Infect. Immun. **57:**1582–1589.
5. **Ebersole, J. L., M.-N. Sandoval, M. J. Steffen, and D. Cappelli.** 1991. Serum antibody in *Actinobacillus actinomycetemcomitans*-infected patients with periodontal disease. Infect. Immun. **59:**1795–1802.
6. **Ebersole, J. L., M. A. Taubman, D. J. Smith, R. J. Genco, and D. E. Frey.** 1982. Human immune responses to oral microorganisms. I. Association of localized juvenile periodontitis (LJP) with serum antibody responses to *Actinobacillus actinomycetemcomitans*. Clin. Exp. Immunol. **47:**43–52.
7. **Ebersole, J. L., M. A. Taubman, D. J. Smith, B. F. Hammond, and D. E. Frey.** 1983. Human immune responses to oral microorganisms. II. Serum antibody responses to antigens from *Actinobacillus actinomycetemcomitans* and the correlation with localized juvenile periodontitis. J. Clin. Immunol. **3:**321–331.
8. **Farida, R., M. Wilson, and L. Ivanyi.** 1986. Serum IgG antibodies to lipopolysaccharides in various forms of periodontal disease in man. Arch. Oral Biol. **31:**711–715.
9. **Granoff, D. M., J. P. Pandey, E. Boies, J. Squires, R. S. Munson, Jr., and B. Suarez.** 1984. Response to immunization with *Haemophilus influenzae* type b polysaccharide-pertussis vaccine and risk of *Haemophilus* meningitis in children with the Km(1) immunoglobulin allotype. J. Clin. Invest. **74:**1708–1714.
10. **Granoff, D. M., B. K. Suarez, J. P. Pandey, and P. G. Shackelford.** 1988. Genes associated with the G2m(23) immunoglobulin allotype regulate the IgG subclass responses to *Haemophilus influenzae* type b polysaccharide vaccine. J. Infect. Dis. **157:**1142–1149.
11. **Gunsolley, J. C., J. G. Tew, C. M. Gooss, J. A. Burmeister, and H. A. Schenkein.** 1988. Effects of race and periodontal status on antibody reactive with *Actinobacillus actinomycetemcomitans* strain Y4. J. Periodontal Res. **23:**303–307.
12. **Hamilton, R. G.** 1987. Human IgG subclass measurements in the clinical laboratory. Clin. Chem. **33:**1707–1725.
13. **Hamilton, R. G.** 1990. Engineered human antibodies as immunologic quality control reagents. Ann. Biol. Clin. **48:**473–477.
14. **Hamilton, R. G.** 1991. Application of engineered chimeric antibodies to the calibration of human antibody standards. Ann. Biol. Clin. **49:**242–248.
15. **Hamilton, R. G., R. W. Wilson, T. Spillman, and M. Roebber.** 1988. Monoclonal antibody-based immunoenzymetric assays for quantification of human IgG and its four subclasses. J. Immunoassay **9:**275–296.
16. **Hammarström, L., and C. I. Smith.** 1986. IgG subclasses in bacterial infections. Monogr. Allergy **19:**122–133.
17. **Hammarström, L., and C. I. Smith.** 1986. IgG subclass changes in response to vaccination. Monogr. Allergy **19:**241–252.
18. **Jefferies, R., C. B. Riemer, F. Skvaril, G. de Lange, and N. R. Ling.** 1985. Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of an IUIS/WHO collaborative study. Immunol. Lett. **10:**223–225.
19. **Morell, A., F. Skvaril, A. Steinberg, E. van Loghem, and W. Terry.** 1972. Correlation between the concentrations of the four

subclasses of IgG and Gm allotypes in normal human sera. J. Immunol. **108:**195–206.

20. **Page, R. C., T. J. Sims, L. D. Engel, B. J. Moncla, B. Bainbridge, J. Stray, and R. P. Darveau.** 1991. The immunodominant outer membrane antigen of *Actinobacillus actinomycetemcomitans* is located in the serotype-specific high-molecular-mass carbohydrate moiety of lipopolysaccharide. Infect. Immun. **59:**3451–3462.
21. **Papadea, C., I. J. Check, and C. B. Reimer.** 1985. Monoclonal antibody-based solid-phase immunoenzymometric assays for quantifying human immunoglobulin G and its subclasses in serum. Clin. Chem. **31:**1940–1945.
22. **Sarvas, H., N. Rautonen, and O. Mäkelä.** 1991. Allotype-associated differences in concentrations of human IgG subclasses. J. Clin. Immunol. **11:**39–45.
23. **Sarvas, H., N. Rautonen, S. Sipinen, and O. Mäkelä.** 1989. IgG subclasses of pneumococcal antibodies—effect of allotype G2m(n). Scand. J. Immunol. **29:**229–237.
24. **Slots, J., H. S. Reynolds, and R. J. Genco.** 1980. *Actinobacillus actinomycetemcomitans* in human periodontal disease: a cross-sectional microbiological investigation. Infect. Immun. **29:**1013–1020.
25. **Spiegelberg, H. L.** 1974. Biological activities of immunoglobulins of different classes and subclasses. Adv. Immunol. **19:**259–294.
26. **Steinberg, A., A. Morell, S. Frantisek, and E. van Loghem.** 1973. The effect of Gm(23) on the concentration of IgG2 and IgG4 in normal human serum. J. Immunol. **110:**1642–1645.
27. **Steinberg, A. G., and C. E. Cook.** 1981. The distribution of the human immunoglobulin allotypes. Oxford University Press, New York.
28. **Tsai, C.-C., W. P. McArthur, P. C. Baehni, C. Evian, R. J. Genco, and N. S. Taichman.** 1981. Serum neutralizing activity against *Actinobacillus actinomycetemcomitans* leukotoxin in juvenile periodontitis. J. Clin. Periodontol. **8:**338–348.
29. **Waldrop, T., B. Mackler, and P. Schur.** 1981. IgG and IgG subclasses in human periodontosis (juvenile periodontitis). J. Periodontol. **52:**96–98.
30. **Watanabe, H., P. D. Marsh, and L. Ivanyi.** 1989. Antigens of *Actinobacillus actinomycetemcomitans* identified by immunoblotting with sera from patients with localized human juvenile periodontitis and generalized severe periodontitis. Arch. Oral Biol. **34:**649–656.
31. **Wilson, M. E.** 1991. IgG antibody response of localized juvenile periodontitis patients to the 29 kilodalton outer membrane protein of *Actinobacillus actinomycetemcomitans*. J. Periodontol. **62:**211–218.
32. **Wilson, M. E., and R. E. Schifferle.** 1991. Evidence that the serotype b antigenic determinant of *Actinobacillus actinomycetemcomitans* Y4 resides in the polysaccharide moiety of lipopolysaccharide. Infect. Immun. **59:**1544–1551.
33. **Zambon, J. J.** 1985. *Actinobacillus actinomycetemcomitans* in human periodontal disease. J. Clin. Periodontol. **12:**1–20.
34. **Zambon, J. J., J. Slots, and R. J. Genco.** 1983. Serology of oral *Actinobacillus actinomycetemcomitans* and serotype distribution in human periodontal disease. Infect. Immun. **41:**19–27.