Opsonization of *Actinobacillus actinomycetemcomitans* by Immunoglobulin G Antibodies to the O Polysaccharide of Lipopolysaccharide

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Sera of localized juvenile periodontitis (LJP) patients colonized by *Actinobacillus actinomycetemcomitans* serotype b often contain markedly elevated levels of immunoglobulin G (IgG) antibodies to serospecific determinants in the O polysaccharide of lipopolysaccharide (LPS), as well as to outer membrane proteins of this species. IgG antibodies in LJP sera are known to opsonize *A. actinomycetemcomitans* for subsequent phagocytosis and killing by human neutrophils. The objective of this study was to determine whether outer membrane proteins or serospecific determinants in LPS are the primary target for opsonic IgG antibodies in LJP sera. An *A. actinomycetemcomitans* serotype b O-polysaccharide affinity column was constructed and subsequently used to purify LPS-specific IgG antibodies from LJP serum. The affinity-purified anti-LPS IgG antibodies were enriched in content of IgG2 (66.2%, compared with 37.0% in the total IgG fraction) and were immunospecific for *A. actinomycetemcomitans* serotype b LPS. In an opsonophagocytic assay using neutrophils from donors who were homozygous for the H131 allotype of Fcγ receptor IIa (CD32), it was found that LPS-specific IgG antibodies exhibited substantially greater opsonic activity toward *A. actinomycetemcomitans* serotype b than an LJP IgG fraction that was depleted of LPS-reactive antibodies but contained antibodies against outer membrane proteins of this species. The results of this study indicate that serospecific determinants in the O polysaccharide of *A. actinomycetemcomitans* serotype b are a principal target for opsonic antibodies in sera of LJP subjects.

Sera of patients with localized juvenile periodontitis (LJP) often contain elevated levels of immunoglobulin G (IgG) antibodies reactive toward *Actinobacillus actinomycetemcomitans* serotype b (7, 12). These IgG antibodies recognize a diverse array of *A. actinomycetemcomitans* antigens including the RTX leukotoxin (25), as well as lipopolysaccharide (LPS) (8, 23, 30) and outer membrane proteins (28, 29, 32) present in the cell envelope. However, the serotype b antigen is considered the immunodominant antigen of *A. actinomycetemcomitans*. First described as a heat-stable, papain-resistant molecule with carbohydrate-like features (4), the serotype b antigen was reported to be a linear polymer composed of disaccharide units containing L-rhamnose and D-fucose (2). We and others provided evidence that the serotype b antigen is associated with the polysaccharide moiety of LPS (17, 30). More recently, we reported that the serotype b antigen is defined by the O polysaccharide (O-PS) of LPS and is a trisaccharide repeating unit consisting of D-fucose, L-rhamnose, and N-acetyl-D-galactosamine (18).

Given the identity of the LPS O-PS as the serotype-specific antigen of *A. actinomycetemcomitans* and its strong reactivity with IgG antibodies in LJP sera, we sought to define the functional properties of IgG antibodies directed to this surface-accessible structure. The polymorphonuclear neutrophil is considered to play a key role in host defense against periodontopathic bacteria (9, 15, 26). Efficient phagocytosis and subsequent intracellular killing of bacteria often require opsonization by specific antibody and/or complement. LJP sera have been found to contain IgG antibodies capable of supporting

phagocytosis and killing of *A. actinomycetemcomitans* serotype b in the presence of complement (3). However, the antigenic specificity of these opsonic IgG antibodies has not heretofore been defined. Accordingly, the objective of this study was to examine the role of O-PS-directed IgG antibodies in promoting phagocytosis and killing of *A. actinomycetemcomitans* by human neutrophils. O-PS-specific IgG antibodies were prepared from LJP serum by affinity chromatography. The opsonic activity of these antibodies was compared with that of an IgG fraction that was depleted of O-PS-specific antibody but contained antibodies to *A. actinomycetemcomitans* outer membrane proteins. The results of this study indicate that IgG antibodies which recognize serospecific determinants in the O-PS of *A. actinomycetemcomitans* serotype b play a major role in opsonization of this species.

MATERIALS AND METHODS

Patient serum. Serum containing elevated levels of IgG antibodies to *A. actinomycetemcomitans* serotype b LPS, as well as to several outer membrane proteins, was obtained from a patient with LJP after informed consent was obtained. The IgG2 fraction of this patient's serum had previously been found to exhibit significant opsonic antibody activity (33). Serum was also obtained from a patient with common variable hypogammaglobulinemia (CVH) and was used as a complement source in bactericidal assays. The CVH serum contained markedly depressed concentrations of IgG, IgA, and IgM but normal levels of complement proteins C3 and C4 and a normal 50% hemolytic complement value.

Isolation of LPS and O-PS. *A. actinomycetemcomitans* ATCC 43718 (strain Y4) was maintained on 5% sheep blood agar as described previously (3). Broth cultures (~26 liters) were prepared in NIH thioglycolate broth (Difco, Detroit, Mich.) supplemented with 5 g of yeast extract and 1 g of sodium bicarbonate per liter and incubated at 37°C in humidified 5% CO₂. The organisms were harvested by centrifugation, washed in phosphate-buffered saline (PBS; pH 7.2), and mixed with 1% (wt/wt) formalin. LPS was extracted into hot aqueous phenol as described previously (30), dialyzed against distilled H₂O to remove phenol, and lyophilized. A 1-mg/ml (wt/vol) solution of *A. actinomycetemcomitans* Y4 LPS was prepared in 1.5% acetic acid (vol/vol) and incubated for 2 h at 100°C to liberate polysaccharide. Insoluble lipid A was removed by centrifugation and filtration (0.45-μm-pore-size filter). Following dialysis against distilled H₂O by

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using a Spectra/Por membrane (molecular weight cutoff, 3,500; Spectrum Medical Industries, Inc., Laguna Hills, Calif.), the lipid-free polysaccharide was lyophilized. The O-PS was further resolved on a Sephadex G-50 column (1.5 by 62 cm) equilibrated with 50 mM pyridylacetic acid (pH 4.5). Collected fractions (~4 ml) were monitored for neutral sugar by the phenol-sulfuric acid method (6). The included volume peak was retained, dialyzed against distilled H₂O (see above), and lyophilized.

Preparation of O-PS affinity column. O-PS (27 mg) was suspended in 4 ml of 0.15 M borate (pH 8.5). Two milliliters of the same buffer, containing 58 mg of 1,3-diaminopropane dihydrochloride and 3 mg of sodium cyanoborohydride (both from Sigma Chemical Co., St. Louis, Mo.), was then added to the O-PS in dropwise manner with constant stirring. The reaction mixture was incubated for 6 days at 45°C. The reaction product was applied to a Bio-Gel P2 column (1.5 by 70 cm) and eluted in 20 mM pyridylacetic acid (pH 5.4). Fractions (~4 ml) were monitored for neutral sugar by the phenol-sulfuric acid method (see above). Polysaccharide-containing fractions were pooled, dialyzed against distilled H₂O, and lyophilized.

CH-Sepharose (1.2 g [dry weight]; Sigma) was sequentially washed in 1 mM HCl, distilled H₂O, and then coupling buffer (0.1 M sodium bicarbonate [pH 7.8] containing 0.5 M sodium chloride). Diaminopropane-derivatized O-PS (27 mg) dissolved in 5.0 ml of coupling buffer was added to the washed gel and mixed end-over-end for 2 h at ambient temperature. The resin was subsequently washed in coupling buffer and then blocked by incubation in 1 M Tris (pH 9.0) with end-over-end rotation for 1 h at room temperature. The gel was then washed sequentially with 10 volumes of 0.15 M NaCl in 50 mM Tris-HCl (pH 8.0) and 10 volumes of 0.5 M NaCl in 10 mM sodium acetate (pH 4.5) as described by Altman and Bundle (1). The O-PS affinity column thus prepared (1.0 by 2.5 cm) was equilibrated with PBS (pH 7.2) and stored at 4°C.

Affinity purification of O-PS-specific IgG antibodies. A gamma globulin fraction of LJP serum containing elevated levels of IgG antibody to *A. actinomycetemcomitans* Y4 LPS was prepared by ammonium sulfate precipitation, followed by ion-exchange chromatography on DEAE-Sephacel (Pharmacia, Uppsala, Sweden). Purity was confirmed by immunoelectrophoresis using anti-whole human serum. The IgG fraction was dialyzed against PBS and stored in aliquots at -70°C.

Four hundred microliters of IgG (3.1 mg/ml) was applied to the O-PS affinity column. Once the sample had entered the gel, 200 µl of PBS was added to facilitate entry of the IgG into the gel matrix, after which the column was sealed and allowed to incubate with the IgG for 60 to 90 min at ambient temperature. Unadsorbed IgG was collected, after which the column was rinsed with PBS until the optical density at 280 nm returned to baseline levels. Bound IgG was subsequently eluted with 0.1 M glycine (pH 2.8) into tubes containing 50 µl of 1.0 M Tris (pH 9.5) per 2 ml of effluent. Elution of IgG was monitored spectrophotometrically at A₂₈₀. Fractions containing adsorbed and unadsorbed IgG were pooled, dialyzed against PBS, and concentrated by pervaporation. The samples were then dialyzed against PBS by using a Pierce Slide-A-Lyzer (molecular weight cutoff, 10,000; Pierce Chemical Co., Rockford, Ill.). Protein concentration was determined by A₂₈₀.

Determination of IgG subclass distribution. Distribution of IgG subclasses in the DEAE IgG, anti-LPS, and anti-LPS-depleted fractions was determined by means of an immunoenzymetric assay using subclass-specific monoclonal antibodies and WHO 67/97 serum as the reference standard, as described elsewhere (11). The results are expressed as a percentage of the total IgG, which was determined by summation of the concentrations of IgG1 to IgG4. The summated values agreed to within 15% of the total IgG concentration as determined independently by radial immunodiffusion.

IgG subclass ELISA. IgG1 and IgG2 antibodies to *A. actinomycetemcomitans* serotype b LPS were detected in various IgG fractions by using an IgG subclass-specific enzyme-linked immunosorbent assay (ELISA), as described previously (31).

Double immunodiffusion. Double-immunodiffusion analysis of LPS prepared by phenol-water extraction of *A. actinomycetemcomitans* 75, Y4, and 67 (representing serotypes a, b, and c, respectively) was performed in 1% agarose in PBS. Peripheral wells contained LPS (1 mg/ml) which was solubilized via addition of 50 µl of triethylamine per ml of LPS solution. The center well contained affinity-purified IgG antibodies (2.6 mg/ml) to the O-PS of *A. actinomycetemcomitans* Y4, as described above.

Immunoblot analysis. Antigenic reactivity of the total IgG, affinity-purified anti-O-PS IgG, and affinity-depleted IgG fractions was assessed by immunoblot analysis. Briefly, high-molecular-mass LPS and outer membrane complex of *A. actinomycetemcomitans* Y4, prepared as described previously (29, 30), were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (30). Electrophoretic transfer of the LPS and outer membrane antigens onto polyvinylidene difluoride (PVDF) membranes (0.45-µm pore size; Immobilon-P; Millipore, Bedford, Mass.) was performed with a semidry electrobloetter (Janssen Life Sciences Products, Piscataway, N.J.) according to the manufacturer's instructions. Following transfer, the PVDF membranes were blocked for 45 min in 1% bovine serum albumin (BSA; fatty acid free) in 20 mM Tris (pH 7.5) containing 0.5 M NaCl (Tris-buffered saline [TBS]), then washed for 5 min in TBS with 0.2% Tween 20, and finally washed with TBS. The membranes were subsequently incubated overnight at 4°C with the specified IgG fraction (100 to 150 µg/ml in TBS with 1% BSA). Following sequential rinsing with TBS-Tween

20 and TBS, the membranes were incubated for 1 h at ambient temperature with a 1:1,000 dilution of biotinylated goat anti-human IgG (γ chain specific; Calbiochem, La Jolla, Calif.) in TBS-1% BSA. The blots were rinsed and incubated for 1 h at ambient temperature with a 1:1,000 dilution of avidin-conjugated alkaline phosphatase (Calbiochem) and then rinsed and developed with 5-bromo-4-chloro-3-indolylphosphate-nitroblue tetrazolium (BCIP-NBT; Sigma Fast; Sigma).

Neutrophil opsonophagocytic assay. Opsonophagocytosis of *A. actinomycetemcomitans* Y4 was determined as described previously (33), using neutrophils from donors who were homozygous for the H131 allele of Fcγ receptor IIa (FcγRIIA) (which binds human IgG2 efficiently). The assays were performed in polypropylene microcentrifuge tubes (Sarstedt, Inc., Newton, N.C.) containing 2×10^6 neutrophils, 3×10^6 to 5×10^6 bacterial CFU of *A. actinomycetemcomitans*, the indicated IgG fraction, 5% (vol/vol) CVH serum as a source of complement, and complete Dulbecco's PBS in a final volume of 0.4 ml. Reactions were initiated via addition of bacteria and incubated at 37°C in humidified 5% CO₂ with continuous end-over-end rotation (approximately 10 to 15 rpm). At time zero and 90 min after addition of the bacterial inoculum, 50 µl samples were withdrawn from the reaction tubes and transferred to tubes containing 4.95 ml of sterile H₂O with 0.2% BSA filter sterilized. Following 1 min of incubation at ambient temperature (in order to lyse neutrophils), 50 µl of the lysate was removed and diluted into 3 ml of sterile half-strength brain heart infusion broth (Difco). Ten-microliter aliquots of this dilution were plated in triplicate onto Trypticase soy agar (Northeast Laboratory, Waterville, Maine). The plates were incubated at 37°C in humidified 5% CO₂ for approximately 72 h, after which CFU per plate was enumerated. Controls included *A. actinomycetemcomitans* Y4 incubated with neutrophils in the presence of (i) heat-inactivated 5% CVH serum (56°C, 30 min), (ii) native 5% CVH serum alone, or (iii) immunoglobulin and 5% heat-inactivated CVH serum. Additional controls included bacteria incubated with immunoglobulin and native or heat-inactivated CVH serum in the absence of neutrophils. The results are expressed as a percentage of the starting (time zero) inoculum.

RESULTS

Affinity purification of anti-serotype b LPS. The serotype b antigen of *A. actinomycetemcomitans* has recently been identified as the O-PS of LPS (18). To evaluate the biologic properties of IgG antibodies to this key surface antigen, we isolated the O-PS from *A. actinomycetemcomitans* serotype b LPS by acetic acid hydrolysis and subsequent chromatography on Sephadex G-50. As described previously in detail (18), this fraction contains the neutral sugars D-fucose and L-rhamnose, as well as N-acetyl-D-galactosamine, in a ratio of 1:1:1. The O-PS was derivatized with a diaminopropane spacer and subsequently coupled to CH-Sepharose. This affinity absorbent was used to isolate IgG antibodies from LJP serum which are specific for the *A. actinomycetemcomitans* serotype b antigen. Serologic specificity of IgG antibodies bound to the O-PS affinity column was confirmed by double-immunodiffusion analysis. As shown in Fig. 1, affinity-purified IgG specific for the O-PS of serotype b LPS exhibited immunoreactivity toward LPS from serotype b but not toward LPS from representative serotype a or c strains. The affinity-purified IgG was similarly unreactive toward LPS derived from strains representing serotypes d and e (not shown). These results are consistent with evidence indicating that the O-PSs of *A. actinomycetemcomitans* serotypes a to e are structurally distinct (18, 19).

Immunoblot analysis. Antigenic specificity of the anti-LPS IgG was further characterized by immunoblot analysis using high-molecular-mass serotype b LPS and Sarkosyl-insoluble outer membrane complexes (containing LPS and outer membrane proteins) as test antigens. High-molecular-weight serotype b LPS electroblotted onto PVDF membranes produced a diffuse smear pattern when probed with the DEAE IgG fraction of high-titer LJP serum (Fig. 2). This smearing is characteristic of the serotype b antigen of *A. actinomycetemcomitans* (4, 17, 30). O-PS-specific anti-LPS IgG prepared by affinity chromatography produced a similar smear pattern, while IgG depleted of O-PS-specific IgG showed minimal immunoreactivity with high-molecular-mass LPS.

Immunoblot analysis performed using outer membrane

FIG. 1. Double-immunodiffusion analysis of affinity-purified anti-LPS IgG from LJP serum. The center well contained affinity-purified IgG (2.6 mg/ml) specific for the O-PS of *A. actinomycetemcomitans* serotype b LPS. Peripheral wells contained LPS (1 mg/ml) isolated from *A. actinomycetemcomitans* serotypes a, b, and c, respectively.

complexes of *A. actinomycetemcomitans* demonstrated that the DEAE fraction of LJP IgG contained antibodies to a number of outer membrane proteins of this species, as well as to LPS. Depletion of O-PS-specific antibodies did not alter immunoreactivity toward *A. actinomycetemcomitans* outer membrane proteins. However, the anti-LPS-depleted IgG no longer produced a smear pattern when reacted with electroblotted outer membrane complexes.

IgG1 and IgG2 antibodies in anti-LPS IgG. High-titer LJP sera contain significantly elevated levels of IgG1 and IgG2 antibodies to *A. actinomycetemcomitans* serotype b LPS (31). Affinity purification of O-PS-specific IgG from LJP serum should, therefore, result in an enrichment of IgG1 and IgG2 antibodies to *A. actinomycetemcomitans* serotype b LPS. Using

FIG. 2. Immunoblot analysis of unadsorbed DEAE IgG, affinity-purified anti-LPS IgG (α -LPS), and IgG depleted of anti-LPS antibody (α -LPS depleted). High-molecular-weight (HMW) LPS and Sarkosyl-insoluble outer membrane complexes were isolated from *A. actinomycetemcomitans* strain Y4 (serotype b), resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subsequently transferred to PVDF membranes. The electroblotted antigens were then reacted with (i) DEAE fraction of high-titer LJP serum, (ii) anti-LPS, or (iii) anti-LPS-depleted IgG prepared from the DEAE fraction by affinity chromatography. IgG binding was visualized by sequential incubation with biotinylated goat anti-human IgG, avidin-conjugated alkaline phosphatase, and enzyme substrate (BCIP-NBT).

FIG. 3. Immunoreactivities of unadsorbed LJP IgG, affinity-purified anti-LPS IgG, and anti-LPS-depleted IgG from high-titer LJP serum toward *A. actinomycetemcomitans* serotype b LPS. Serotype b LPS-reactive IgG1 (A) and IgG2 (B) antibodies were detected by ELISA, using human IgG subclass-specific monoclonal antibodies as described previously (31).

an IgG subclass-specific ELISA, we assessed the content of LPS-reactive IgG1 and IgG2 antibodies in the affinity-purified anti-LPS IgG. As shown in Fig. 3A, IgG1 antibody activity toward serotype b LPS was greater in the anti-LPS IgG fraction than in the DEAE IgG fraction, while IgG depleted of LPS-reactive antibody contained minimal IgG1 antibody activity toward LPS. Similarly, affinity-purified anti-LPS IgG showed enrichment of IgG2 antibody activity toward serotype b LPS (Fig. 3B).

IgG subclass distribution of anti-LPS antibodies. Polysaccharide antigens typically induce the production of IgG antibodies which are principally of the IgG2 subclass, although IgG1 antibodies are often present. Using a human IgG subclass-specific ELISA, we previously observed that sera of LJP patients contain substantial concentrations of IgG2 antibodies to *A. actinomycetemcomitans* serotype b LPS (31). We sought independent confirmation of this finding by examining the subclass distribution of affinity-purified IgG antibodies to the O-PS of *A. actinomycetemcomitans* serotype b. As shown in

TABLE 1. Subclass distribution of total IgG, affinity-purified anti-LPS IgG, and anti-LPS-depleted IgG prepared from LJP serum

Fraction	% of total IgG ^a			
	IgG1	IgG2	IgG3	IgG4
Total (DEAE) IgG ^b	50.6	37.0	6.5	5.9
Affinity-purified anti-LPS IgG	26.5	66.2	0.0	7.3
Anti-LPS-depleted IgG	58.0	33.8	5.9	2.3

^a Subclass concentrations were determined by enzyme immunoassay (31). Total IgG was calculated by summation of the IgG1 through IgG4 subclass concentrations. Subclass distribution is expressed as a percentage of the total IgG concentration in each fraction.

^b Prepared from high-titer LJP serum by ammonium sulfate precipitation and subsequent ion-exchange chromatography on DEAE-Sephacel.

Table 1, affinity-purified IgG specific for the O-PS of serotype b LPS was enriched in content of IgG2 (66.2% of total) compared with the unadsorbed (37.0% of total) DEAE IgG fraction of LJP serum. On the other hand, the proportion of IgG1 in the anti-LPS IgG was nearly 50% lower than that found in the unadsorbed IgG and was slightly increased in the IgG fraction depleted of LPS-reactive antibody. These results provide further evidence that IgG antibodies directed to serospecific determinants of *A. actinomycetemcomitans* serotype b LPS are principally of the IgG2 subclass.

Opsonic activity of anti-LPS antibodies. LJP sera contain opsonic IgG antibodies capable of facilitating phagocytosis and killing of *A. actinomycetemcomitans* by human neutrophils (3). We sought to determine whether these opsonic IgG antibodies recognize LPS or outer membrane proteins of *A. actinomycetemcomitans*. Accordingly, we compared the abilities of affinity-purified anti-LPS IgG and IgG depleted of anti-LPS antibody to support phagocytosis and killing of *A. actinomycetemcomitans* serotype b by neutrophils. The anti-LPS-depleted IgG contained antibodies reactive toward a number of outer membrane proteins of this strain (Fig. 2). Given that the affinity-purified anti-LPS IgG was highly enriched in content of IgG2, we used neutrophils from donors who were homozygous for the H131 allele of FcγRIIa (CD32), the only FcγR known to bind human IgG2 efficiently (27, 34). As depicted in Fig. 4, anti-LPS IgG exhibited substantially greater opsonic activity than anti-LPS-depleted IgG during a 90-min incubation period. In the presence of complement, anti-LPS IgG exhibited significant opsonic activity (>90% loss of bacterial viability) at concentrations of <1 μg/ml. In contrast, opsonic activity of the anti-LPS-depleted IgG fraction became manifest only at concentrations exceeding 25 μg/ml in the presence of complement. Neither anti-LPS IgG nor anti-LPS-depleted IgG expressed opsonic activity in the presence of heat-inactivated complement, nor was opsonic activity observed in the presence of complement alone. Moreover, incubation of *A. actinomycetemcomitans* Y4 with anti-LPS IgG (50 μg/ml) in the presence of native 5% CVH serum yielded no loss of bacterial viability after 90 min (188.8% of the time zero inoculum), indicating that the anti-LPS IgG lacked bactericidal antibody activity.

DISCUSSION

LJP patients are frequently colonized by serotype b strains of *A. actinomycetemcomitans* (30). Moreover, LJP sera often contain significantly elevated levels of IgG antibodies directed to the serotype b-specific antigen (4, 23). This antigen has been found to be associated with the polysaccharide region of LPS (17, 30). In particular, structural analysis has revealed that the

serotype b antigen is defined by a trisaccharide repeating unit containing D-fucose, L-rhamnose, and N-acetyl-D-galactosamine in the O-PS of LPS (18). The O-PSs of serotypes a through e have distinct antigenic structures, while the core polysaccharide and lipid A structures are conserved among these five serotypes (19).

Information pertaining to the influence of humoral immunity upon the dynamics of periodontal infection caused by *A. actinomycetemcomitans* serotype b is limited. Previous studies indicating that fewer teeth are involved in LJP patients with precipitating serum IgG antibodies to *A. actinomycetemcomitans* (20) and that serum IgG antibody titer is inversely correlated with attachment loss (10) suggest that IgG antibodies against *A. actinomycetemcomitans* may be protective. More recently, it was reported that generalized early-onset periodontitis patients with high levels of IgG antibody to *A. actinomycetemcomitans* serotype b LPS exhibited significantly less attachment loss than patients with low to moderate anti-LPS antibody levels (5). Hence, antibodies directed to LPS-associated determinants may contribute to host defense against *A. actinomycetemcomitans* infection.

The O-PSs of gram-negative bacteria are immunologically important structures due to their surface accessibility (8, 14, 21, 22, 28). Monoclonal and polyclonal antibodies directed to LPS O-PSs have been demonstrated to protect against experimental infections caused by certain species of gram-negative bacteria and to promote their opsonization in vitro (16, 24). Monoclonal antibodies to the serotype b antigen of *A. actinomycetemcomitans* have been shown to promote killing of this organism by human neutrophils in the presence of complement (35). IgG antibodies present in sera of LJP patients also promote phagocytosis and killing of *A. actinomycetemcomitans*

FIG. 4. Comparison of the opsonic activities of affinity-purified anti-LPS (serotype b) IgG and IgG depleted of LPS-reactive antibody. Anti-LPS (O-PS-specific) IgG was prepared from high-titer LJP IgG by affinity chromatography on CH-Sepharose coupled to the O-PS of *A. actinomycetemcomitans* serotype b via a diaminopropane spacer. Unbound material was collected and used as a source of anti-LPS-depleted IgG. *A. actinomycetemcomitans* serotype b (strain Y4) and neutrophils (from an individual homozygous for the H131 allele of FcγRIIa) were incubated for 90 min in the presence of 5% CVH serum (as a complement source). Viability is expressed as a percentage of the starting inoculum. The results depicted are from a single experiment and are representative of those obtained in three such experiments.

serotype b by human neutrophils (3), although the antigenic specificity of these opsonic antibodies was not defined.

High-titer LJP sera contain IgG antibodies directed to serospecific determinants in LPS, as well as to outer membrane proteins of *A. actinomycetemcomitans*. The focus of the current study was to determine the role of IgG antibodies directed to serospecific determinants in the O-PS moiety of LPS in facilitating phagocytosis and killing of *A. actinomycetemcomitans* serotype b by human neutrophils. Accordingly, anti-LPS IgG antibodies, specific for the O-PS of serotype b LPS, were isolated from high-titer LJP serum by means of affinity chromatography. IgG antibodies eluted from the O-PS affinity column exhibited serotype specificity, reacting with LPS from serotype b but not from serotype a or c (Fig. 1). Moreover, these antibodies, when reacted with electroblooded serotype b LPS, produced a smear pattern characteristic of the serotype b antigen. IgG antibodies depleted of O-PS-reactive antibody failed to produce this smear but were reactive toward a number of *A. actinomycetemcomitans* outer membrane proteins (Fig. 2).

IgG antibodies specific for the O-PS were found to be enriched in content of IgG1 and IgG2 antibodies to serotype b LPS (Fig. 3). As determined by an IgG subclass-specific immunoenzymetric assay, the affinity-purified antibodies contained 66.2% IgG2 and 26.5% IgG1. In contrast, the DEAE IgG fraction from which the anti-O-PS antibodies were isolated contained 37.0% IgG2 and 50.6% IgG1. These results provide further evidence that IgG antibodies directed to serospecific determinants in *A. actinomycetemcomitans* LPS are primarily of the IgG2 subclass (13, 31).

Given that sera of high-titer LJP patients contain substantial amounts of IgG2 antibodies directly not only to LPS-associated determinants but also to at least one major outer membrane protein (32), we previously examined the opsonic activity of an IgG2 subclass-restricted fraction of LJP serum. When used in conjunction with complement and neutrophils expressing the H131 allotype of Fc γ R1a, which binds human IgG2 efficiently, LJP IgG2 was highly opsonic for *A. actinomycetemcomitans* serotype b, whereas nonimmune IgG2 lacked such activity (33). Affinity-purified IgG1 from this same serum was also opsonic and synergized with the IgG2 fraction in supporting killing of *A. actinomycetemcomitans*. These results indicated that both IgG1 and IgG2 antibodies in LJP serum express opsonic activity toward this organism, although the antigenic specificity of these antibodies was not examined.

The results of the present study indicate that IgG antibodies to serospecific determinants in LPS play a greater role in supporting opsonophagocytosis of *A. actinomycetemcomitans* than do IgG antibodies which recognize outer membrane proteins of this species. Given that (i) IgG2 antibodies are opsonic for *A. actinomycetemcomitans*, (ii) LPS-specific IgG antibodies belong chiefly to this subclass, and (iii) affinity-purified anti-O-PS IgG contains 2.5 times as much IgG2 as IgG1, it is tempting to hypothesize that the opsonic activity of IgG specific for the O-PS of serotype b LPS is largely attributable to antibodies of the IgG2 subclass. To test this hypothesis, we are currently preparing IgG1 and IgG2 subclass-restricted fractions of anti-O-PS IgG and will assess the opsonic activity of each. Moreover, we will examine the influence of the H131/R131 polymorphism of Fc γ R1a upon the expression of opsonic activity by IgG2 antibodies specific for the O-PS of *A. actinomycetemcomitans*.

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