Immunoglobulin G2 Antibodies Promote Neutrophil Killing of *Actinobacillus actinomycetemcomitans*

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Sera from patients with localized juvenile periodontitis (LJP) often contain markedly elevated levels of immunoglobulin G2 (IgG2) antibodies reactive to cell envelope constituents of *Actinobacillus actinomycetemcomitans*. The objective of this study was to determine if these IgG2 antibodies are capable of supporting phagocytosis and killing of *A. actinomycetemcomitans* by human neutrophils. Polyclonal IgG2 antibodies were prepared from high-titer LJP serum by affinity chromatography, yielding a preparation which was >99% subclass restricted and retained immunoreactivity to *A. actinomycetemcomitans* antigens. Affinity-purified IgG2 antibodies were evaluated by an in vitro opsonophagocytic assay that employed neutrophils obtained from donors who were homozygous for the H131 allotype of Fcγ receptor type IIa (CD32), which efficiently binds human IgG2 antibodies. Affinity-purified IgG2 antibodies from LJP serum but not from sera of periodontally healthy individuals promoted phagocytosis and killing of *A. actinomycetemcomitans*. The expression of IgG2-dependent opsonic activity required the presence of complement. Incubation of *A. actinomycetemcomitans* with neutrophils in the presence of an optimal concentration of LJP IgG2 (50 μg/ml) and 5% hypogammaglobulinemic serum (as a complement source) resulted in a >1 log₁₀ reduction in bacterial viability within 30 min. The opsonic activity of IgG2 antibodies was found to be comparable to that observed with affinity-purified IgG1 antibodies. Moreover, IgG1 antibodies interacted synergistically with IgG2 antibodies in promoting opsonophagocytosis of *A. actinomycetemcomitans*. The results of this study indicate that LJP serum contains IgG2 antibodies which, when employed in conjunction with neutrophils that express Fcγ receptors capable of recognizing this subclass, are opsonic for *A. actinomycetemcomitans*.

Actinobacillus actinomycetemcomitans is a gram-negative coccobacillus which has been associated with localized juvenile periodontitis (LJP) (38). Sera from LJP patients often contain markedly elevated immunoglobulin G (IgG) antibody titers against *A. actinomycetemcomitans* antigens, including RTX leukotoxin, serospecific determinants in lipopolysaccharide (LPS), and outer membrane proteins (OMPs) (8, 28, 34, 35, 37). Although elevated IgG antibody titers to this organism are thought to limit disease severity and promote localization of infection in LJP (11, 23), information regarding the mechanism(s) of humoral immunity and the antigenic specificity of protective antibody is limited.

Sera from healthy individuals contain four distinct subclasses of IgG which differ in relative concentrations, physical and biologic properties, affinity for certain antigens, and the nature of the antigens which appear to elicit their production (9, 14, 15, 22, 26). IgG2 antibodies are reportedly less effective than either IgG1 or IgG3 antibodies in activating complement via the classical pathway and in binding to Fc receptors for IgG (FcγRs) expressed on mononuclear and polymorphonuclear phagocytes. Nevertheless, patients who are selectively deficient in the ability to produce IgG2 antibodies to certain carbohydrate antigens often exhibit increased susceptibility to recurrent bacterial infection (16), which is indicative of the importance of this IgG subclass in host defense.

Recent evidence indicates that IgG2 antibodies predominate in the IgG response of LJP subjects to principal surface antigens of *A. actinomycetemcomitans*, including LPS and at least

one principal OMP (17, 18, 36, 36a). The biologic significance of these IgG2 antibodies has not been defined. We previously reported that LJP sera contain IgG antibodies capable of promoting phagocytosis and killing of *A. actinomycetemcomitans* by human neutrophils (4). In this study, we sought to define the role of *A. actinomycetemcomitans*-specific IgG2 antibodies in facilitating neutrophil killing of this organism.

Phagocyte recognition of bacteria opsonized with complement fragments (C3b and iC3b) and/or IgG is a receptor-mediated event that involves membrane receptors for complement (CR1 and CR3) or FcγR. Three main classes of FcγRs on human leukocytes, two of which (FcγRII [CD32] and FcγRIII [CD16]) are constitutively expressed on neutrophils (29), have been identified. Studies defining the structural features of the family of FcγRs have revealed the existence of multiple isoforms within each class. Moreover, a genetically defined polymorphism for FcγRIIa has recently been described and shown to influence the binding of human IgG subclasses (3, 30). One such allotypic form, FcγRIIa-H131 (previously referred to as low responder [LR]), is the only member of the FcγR family known to bind IgG2 efficiently. Phagocytes that express this allotype are much more efficient in ingesting IgG2-coated erythrocytes and bacteria than cells that express the FcγRIIa-R131 (previously termed the high-responder [HR]) allotype (5, 24). These findings prompted our use of neutrophils that expressed the FcγRIIa-H131 allotype as effectors in assays designed to evaluate the opsonic properties of affinity-purified IgG2 antibodies isolated from LJP serum.

MATERIALS AND METHODS

Bacteria. *A. actinomycetemcomitans* Y4 (ATCC 43718) was maintained on 5% sheep blood agar supplemented with 1 g of yeast extract (Difco Laboratories,

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Detroit, Mich.), 1 mg of 3-phytylmenadione, and 5 mg of equine hemin III (Sigma Chemical Co., St. Louis, Mo.) per liter and incubated at 37°C in humidified 5% CO₂. On the day prior to performing each bactericidal assay, 10-ml aliquots of half-strength brain heart infusion broth (Difco) supplemented with 10 g of yeast extract, 5 g of hemin, 1 g of menadione, and 1 g of sodium bicarbonate per liter were inoculated with a loopful of colonies from blood agar plates (~48-h old). Following overnight incubation at 37°C in humidified 5% CO₂, aliquots (0.5 to 1 ml) of the overnight culture were transferred to fresh tubes that contained 10 ml of half-strength brain heart infusion broth. These tubes were subsequently incubated as described above until the cultures attained an optical density at A_{650} of 0.13 (corresponding to approximately 6×10^7 CFU per ml).

Sera. Serum was obtained from a patient with common variable hypogammaglobulinemia (HyS) and employed as a complement source in the bactericidal assays described below. Serum from this patient contained less than 8 mg of IgA per dl (normal range, 70 to 312 mg/dl), 36 mg of IgG per dl (normal range, 600 to 1,700 mg/dl), and 7 mg of IgM per dl (normal range, 56 to 352 mg/dl). Sera were also obtained from healthy adult volunteers with no history of periodontal disease and from LJP subjects who received treatment through the Periodontal Disease Clinical Research Center, State University of New York at Buffalo. Informed consent was obtained from all subjects that participated in this study.

Affinity purification of IgG subclass antibodies. The IgG fraction of pooled healthy human serum or LJP serum was prepared by ammonium sulfate precipitation and subsequent ion-exchange chromatography on DEAE-Sephacel (Pharmacia, Uppsala, Sweden). The immunoglobulin fraction thus obtained is referred to as unfractionated IgG. Monoclonal anti-human IgG1 (HP6069; Calbiochem, La Jolla, Calif.) and anti-human IgG2 (HP6002; Calbiochem) antibodies were isolated from ascites fluid by affinity chromatography on an Affi-Prep protein A cartridge (Bio-Rad, Richmond, Calif.). Monoclonal antibodies were oxidized with sodium *m*-periodate and coupled to a CarboLink gel support (Pierce, Rockford, Ill.) according to the manufacturer's instructions. Approximately 0.6 to 1.2 mg of unfractionated human IgG was applied to an anti-IgG2 column. Following 1 to 2 h of incubation at ambient temperature, unbound protein was removed and the column was rinsed in phosphate-buffered saline (PBS). Bound IgG2 antibodies were eluted with 0.1 M glycine, pH 2.8, during which eluted fractions were immediately neutralized by collection into tubes that contained 1 M Tris, pH 9.5. Following dialysis against PBS, affinity-purified IgG2 antibodies were subsequently applied to an anti-IgG1 column to eliminate IgG1 contaminants. Similarly, affinity-purified IgG1 antibodies were prepared by sequential chromatography on anti-IgG1 and anti-IgG2 columns. The IgG subclass distribution of affinity-purified IgG1 and IgG2 antibodies was determined by immunoenzymetric assay, as described previously (13).

Neutrophil isolation. Neutrophils were obtained from peripheral venous blood of healthy adult volunteers by a modification of a technique described previously (4). Briefly, venous blood was collected into polypropylene tubes that contained disodium EDTA (0.15% [final concentration]), after which polymorphonuclear neutrophils were obtained by differential centrifugation and unit gravity sedimentation for 45 min at 37°C in 2.0% gelatin (Difco) in sterile 0.9% saline. Residual erythrocyte contamination was eliminated by hypotonic lysis, after which leukocytes were rinsed in calcium- and magnesium-free (incomplete) Dulbecco's PBS (GIBCO, Grand Island, N.Y.) (pH 7.4) supplemented with 0.1% D-glucose and 0.2% bovine serum albumin (Miles Diagnostics, Kankakee, Ill.). Neutrophils were subsequently resuspended in complete Dulbecco's PBS (supplemented with 0.1% D-glucose and 0.2% bovine serum albumin), enumerated with a hemacytometer, and adjusted to a density of 10^7 leukocytes per ml for use in opsonophagocytic assays (see below).

Prospective neutrophil donors were evaluated with respect to allelic polymorphism of low-affinity FcγRIIA as described elsewhere (21). Individuals homozygous for the H131 allotype, which efficiently binds human IgG2 antibodies, were employed as neutrophil donors for bactericidal assays designed to assess opsonic activity of IgG2 antibodies in patient and control sera.

Neutrophil opsonophagocytic assay. Bactericidal assays were performed in polypropylene microcentrifuge tubes (Sarstedt, Inc., Newton, N.C.) that contained 2×10^6 neutrophils, 3×10^6 to 5×10^6 bacterial CFU of *A. actinomycetemcomitans* Y4, 5% complement (HyS), IgG (unfractionated or affinity-purified IgG1 or IgG2), and complete Dulbecco's PBS in a final volume of 0.4 ml. Reactions were initiated via the addition of bacteria and incubated at 37°C in humidified 5% CO₂ with continuous end-over-end rotation (at approximately 10 to 15 rpm). At time zero and specified intervals thereafter, 50-μl samples were removed and diluted 100-fold into sterile H₂O that contained 0.2% bovine serum albumin. The tubes were gently mixed for 1 min to effect neutrophil lysis, after which 50-μl aliquots were diluted 60-fold into sterile half-strength brain heart infusion broth. Ten-microliter aliquots 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 were enumerated. In each experiment, controls included bacteria incubated with neutrophils in the presence of (i) heat-inactivated 5% complement (56°C, 30 min), (ii) native 5% complement, or (iii) immunoglobulin and 5% heat-inactivated complement. Preliminary studies indicated that *A. actinomycetemcomitans* Y4 was not killed by complement and either unfractionated IgG, IgG1, or IgG2 antibodies in the absence of neutrophils, consistent with the serum-resistant nature of this organism.

FIG. 1. Immunoreactivities of unfractionated IgG and affinity-purified IgG2 antibodies from LJP serum to *A. actinomycetemcomitans* Y4 LPS. LPS-reactive IgG2 antibodies were detected by ELISA with human IgG subclass-specific monoclonal antibodies, as described previously (36).

RESULTS

Affinity purification of IgG2 antibodies. IgG2 subclass-restricted fractions of LJP serum and pooled serum from periodontally healthy individuals were prepared by affinity chromatography with murine anti-human IgG subclass-specific monoclonal antibodies. Serum from the LJP patient contained elevated levels of IgG2 (7.21 g/liter; normal range, 1.0 to 4.6 g/liter) and total IgG (15.98 g/liter; normal range, 3.8 to 15.0 g/liter). In an IgG subclass-specific enzyme-linked immunosorbent assay (ELISA), this serum was found to contain substantial amounts of IgG2 antibodies reactive to major surface antigens of *A. actinomycetemcomitans*, including LPS and the heat-modifiable 29-kDa OMP.

The purity of IgG2 fractions prepared from healthy and LJP sera was >99%, as determined by IgG subclass-specific immunoenzymetric assays. As depicted in Fig. 1, the affinity-purified IgG2 fraction from LJP serum retained significant immunoreactivity to *A. actinomycetemcomitans* Y4 LPS, as well as to the 29-kDa OMP (36a). Affinity purification of IgG2 antibodies resulted in fourfold enhancement of LPS-specific IgG2 antibody activity per milligram of protein over that observed for unfractionated IgG, as determined by quantitative IgG subclass-specific ELISA. Affinity-purified IgG2 antibodies from pooled healthy serum were only weakly reactive to either the *A. actinomycetemcomitans* 29-kDa OMP (36a) or LPS (data not shown).

Opsonic activity of affinity-purified IgG2 antibodies. Unfractionated IgG and affinity-purified IgG2 antibodies from LJP serum were evaluated for the ability to promote phagocytosis and killing of *A. actinomycetemcomitans* by human neutrophils homozygous for the H131 allotype of FcγRIIA. As depicted in Fig. 2, unfractionated IgG from LJP serum exhibited concentration-dependent opsonic activity to *A. actinomycetemcomitans* in the presence of complement (5% HyS). This finding was consistent with previous observations that sera from LJP subjects contain opsonic IgG antibodies to this organism (4). Affinity-purified IgG2 antibodies prepared from this same fraction also expressed considerable opsonic activity. The parallelism observed between the dose-response curves for unfractionated IgG and affinity-purified IgG2 antibodies suggested that much of the opsonic activity of the IgG fraction

FIG. 2. Opsonic activities of unfractionated IgG and affinity-purified IgG2 antibodies from high-titer serum of an LJP subject. *A. actinomycetemcomitans* Y4 was incubated with neutrophils in the presence of 5% complement (H₇S) and various concentrations of either unfractionated IgG or affinity-purified IgG2 antibodies for 90 min. The IgG subclass distribution of unfractionated IgG was as follows: IgG1, 46.8%; IgG2, 42.9%; IgG3, 5.7%; IgG4, 4.6%. Viability is expressed as a percentage of the starting inoculum.

may be attributed to antibodies of the IgG2 subclass. However, as noted below, affinity-purified IgG1 antibodies prepared from the same DEAE fraction of LJP serum also exhibited opsonic activity to *A. actinomycetemcomitans* and may account for the noted difference in the degree of opsonic activity between unfractionated IgG and affinity-purified IgG2 antibodies.

Specificity of IgG2-mediated opsonic activity. To determine if the opsonic activity of the IgG2 fraction prepared from LJP serum resulted from the presence of specific antibodies or was due to nonspecific immunoglobulin activity, we compared the opsonic activities of affinity-purified IgG2 antibodies prepared from LJP and pooled healthy human sera. As shown in Fig. 3, affinity-purified IgG2 antibodies from high-titer LJP serum exhibited measurable opsonic activity at concentrations of 5 to 50 μg/ml. Bacterial viability after 90 min of incubation was reduced by 85% in the presence of 12.5 μg of LJP IgG2 antibodies per ml and by 45% in the presence of 6.25 μg of IgG2 per ml. In contrast, affinity-purified IgG2 antibodies prepared from pooled healthy serum lacked detectable activity over this same concentration range; viability of the organisms after incubation with neutrophils in the presence of 50 μg of nonimmune IgG2 per ml was 156%. Hence, the opsonic activity of the IgG2 fraction of LJP serum appeared to require the presence of specific antibodies.

Kinetics of IgG2-mediated opsonic activity. Next, we examined the kinetics of killing of *A. actinomycetemcomitans* Y4 by neutrophils (homozygous for the H131 allotype of FcγRIIa) in the presence of various opsonins. Serum complement (5% H₇S) failed to support killing of *A. actinomycetemcomitans* during a 2-h incubation (Fig. 4). Affinity-purified IgG2 antibodies (50 μg/ml) from LJP serum exhibited marginal opsonic activity in the presence of heat-inactivated complement. However, incubation of *A. actinomycetemcomitans* with IgG2 antibodies and complement resulted in efficient killing of this organism by neutrophils, with a >1 log₁₀ reduction in viability seen within 30 min. The synergistic effect of IgG2 antibodies and complement was not due to the presence of specific anti-

FIG. 3. Comparison of opsonic activities of affinity-purified IgG2 antibodies isolated from LJP serum and pooled serum from periodontally healthy subjects (nonimmune). Bacteria and neutrophils were incubated for 90 min in the presence of 5% H₇S and various concentrations of immune (LJP) or nonimmune (periodontally healthy) IgG2 antibodies. Viability is expressed as a percentage of the starting inoculum.

bodies in H₇S, as evidenced by the fact that both native H₇S and heat-inactivated H₇S lacked opsonic activity. Moreover, H₇S was initially screened by ELISA and found to contain undetectable antibody activity to an outer membrane fraction of *A. actinomycetemcomitans*.

Comparative opsonic activities of IgG1 and IgG2 antibodies. The LJP serum employed as a source of affinity-purified IgG2 antibodies also contained *A. actinomycetemcomitans*-specific IgG1 antibody activity, as determined by ELISA. Accordingly, we obtained polyclonal IgG1 antibody from the IgG fraction of this serum by affinity chromatography and compared the opsonic activity of this fraction with that of IgG2. As shown in Fig. 5, the opsonic activity of polyclonal IgG1 antibody (>99% subclass restricted) was comparable to that of IgG2 antibody

FIG. 4. Kinetics of neutrophil bactericidal activity to *A. actinomycetemcomitans* Y4 in the presence of various sources of opsonins. Bacteria and neutrophils (homozygous for the LR allotype of FcγRIIa) were incubated for specified intervals in the presence of complement (5% H₇S, native or heat inactivated at 56°C for 30 min) and/or affinity-purified IgG2 antibodies from LJP serum. Viability is expressed as a percentage of the starting inoculum.

FIG. 5. Opsonic activities of affinity-purified IgG1 and IgG2 antibodies from LJP serum. *A. actinomycetemcomitans* Y4 organisms and neutrophils from an H131 homozygous individual were incubated for 60 min at 37°C in the presence of various concentrations of affinity-purified polyclonal IgG1 or IgG2 antibodies and 5% complement (H₂S). Viability is expressed as a percentage of the starting inoculum.

over the range of concentrations tested (3.1 to 25 µg/ml). As with IgG2 antibody, polyclonal IgG1 antibody lacked detectable opsonic activity in the absence of complement.

Synergistic effects of IgG1 and IgG2 antibodies in promoting opsonophagocytosis of *A. actinomycetemcomitans*. IgG1 and IgG2 antibodies isolated from high-titer LJP serum were shown to be nearly equipotent in opsonizing *A. actinomycetemcomitans*. This prompted us to examine the ability of IgG1 and IgG2 antibodies to act in concert in facilitating opsonophagocytosis of this organism. *A. actinomycetemcomitans* Y4 was incubated with neutrophils from an H131 homozygous individual in the presence of 5% H₂S and a suboptimal concentration (3.1 µg/ml) of IgG1 or IgG2 antibodies or a combination thereof and incubated at 37°C for various intervals. As shown in Fig. 6, the addition of both IgG1 and IgG2 antibodies to the opsonophagocytic assay yielded markedly greater killing of *A. actinomycetemcomitans* than was observed with either IgG subclass alone. These results indicate that IgG1 and IgG2 antibodies can function synergistically in supporting opsonization of this organism.

DISCUSSION

Sera from LJP subjects often contain significantly elevated levels of IgG antibodies against *A. actinomycetemcomitans*. The availability of murine monoclonal antibodies specific for each of the four human IgG subclasses has facilitated recent efforts to define the IgG subclass distribution of antibodies to selected *A. actinomycetemcomitans* antigens. Available evidence indicates that IgG antibodies to at least two cell envelope components, LPS and the OmpA-like 29-kDa OMP, are predominantly of the IgG2 isotype (17, 18, 36a). We previously reported that LJP sera contain opsonic IgG antibodies against *A. actinomycetemcomitans* (4). Given the apparent predominance of IgG2 antibodies in LJP sera, we sought to define the role of such antibodies in promoting phagocytosis and killing of *A. actinomycetemcomitans* by human neutrophils. The results of this study indicate that IgG2 antibodies in LJP serum are opsonic for this organism and that complement is required for full manifestation of such activity.

FIG. 6. Synergy between affinity-purified IgG1 and IgG2 antibodies in promoting opsonophagocytosis of *A. actinomycetemcomitans* Y4. Bacteria were incubated with neutrophils obtained from an H131 homozygous donor in the presence of 5% complement alone (H₂S) or suboptimal concentrations (3.1 µg/ml) of IgG1 or IgG2 antibodies or both. Viability is expressed as a percentage of the starting inoculum.

IgG2 antibodies have been considered to be poorly opsonic because of low affinity for FcγRs on phagocytic cells. However, the validity of this conclusion has been questioned in light of recent information that pertains to the structural and functional diversity of the family of human FcγRs. Three main classes of FcγRs, whose genes generate at least 12 different isoforms (including soluble FcγRs), are currently recognized. A genetically defined polymorphism in FcγRIIa was identified during studies to examine T-cell proliferative responses induced by murine anti-CD3 antibodies, a reaction which requires interaction between the antibodies and monocyte FcγRs (27). Monocytes from approximately 70% of white subjects (HRs) were found to support T-cell mitogenic responses induced by murine anti-CD3 antibodies of the IgG1 isotype, whereas monocytes from the remaining 30% of subjects tested did not (LRs).

The HR and LR allotypes of FcγRIIa were shown to differ by only two amino acids (32). Fibroblasts transfected with cDNAs that encoded the HR and LR forms exhibited differential binding of murine and human IgG subclass antibodies, with only the LR form binding human IgG2 antibodies efficiently (31). Moreover, monocytes and neutrophils that express the LR form of FcγRIIa bind and internalize erythrocytes and certain bacteria coated with human IgG2 antibodies more readily than cells that express the HR form (5, 24). A single base substitution (G → A) that results in a change in amino acid residue 131 from arginine (R) to histidine (H) has been shown to be critical for human IgG2 antibody binding to the LR form (33). Accordingly, the HR and LR nomenclature has been replaced by a single letter (H or R) to denote the respective amino acid at position 131 (21).

Given that only neutrophils with the H131 allotype of FcγRIIa bind human IgG2 antibodies efficiently, we utilized neutrophils from donors who were homozygous for this allotype in bactericidal assays designed to assess the opsonic activity of IgG2 antibodies from LJP serum. Individuals with this receptor allotype were identified by a recently described technique involving PCR amplification of genomic DNA and subsequent Southern analysis with allele-specific oligonucleotide probes (21). By employing such neutrophils, IgG2 antibodies

prepared from high-titer LJP serum were found to support efficient killing of *A. actinomycetemcomitans* in the presence of complement (5% H₂S). Bacterial viability was reduced by 45% following incubation for 90 min in the presence of 6.25 µg of LJP IgG2 antibodies per ml and by 85% in the presence of 12.5 µg of LJP IgG2 antibodies per ml. Nonimmune IgG2 antibodies from pooled serum of periodontally healthy subjects lacked detectable opsonic activity at concentrations of up to 50 µg/ml. IgG2-mediated phagocytosis and killing of *A. actinomycetemcomitans* was found to be quite rapid (Fig. 4), with >90% reduction in viability after 30 min in the presence of 50 µg of IgG2 antibodies per ml and complement.

The opsonic activity of affinity-purified IgG2 antibodies was found to be comparable to that of IgG1 antibodies prepared from the same LJP serum. Moreover, IgG1 and IgG2 antibodies were able to act synergistically in promoting opsonization of *A. actinomycetemcomitans*. IgG1 antibodies are able to bind to both forms of low-affinity FcγR (CD32 and CD16) expressed on neutrophils, whereas IgG2 antibodies bind to CD32 only. Whether IgG1 antibodies are able to synergize with IgG2 antibodies through interaction with CD32, CD16, or both has not been defined. Future studies that employ monoclonal antibodies capable of selectively blocking CD16 will enable us to determine the receptor(s) utilized by opsonic IgG1 antibodies to *A. actinomycetemcomitans*.

We have observed that IgG2- and IgG1-mediated opsonic activities are critically dependent on the presence of complement. Whether this is due to direct activation of the alternative pathway by constituents of the outer membrane of *A. actinomycetemcomitans* or to classical pathway activation by IgG1- and/or IgG2-containing immune complexes is unclear. IgG2 antibodies are considered to exhibit only weak classical pathway fixing activity on the basis of studies that employed aggregated myeloma proteins or chimeric antibodies that contained murine variable regions of monoclonal antibodies (7). However, neither of these situations is entirely physiologic. On the other hand, more recent studies with polyclonal IgG2 antibodies directed to diverse bacterial antigens indicate that these antibodies exhibit significant anticomplementary activity, as measured by fixation of C3c (6). In addition, complement has been reported to enhance phagocytosis of IgG2-coated bacteria by neutrophils (2, 5). Our results indicate that opsonization of *A. actinomycetemcomitans* by IgG subclass antibodies is greatly enhanced by complement.

Our results also indicate that IgG2 antibodies to *A. actinomycetemcomitans* can be effective opsonins when they interact with phagocytes that express the H131 allotype of FcγRIIa. However, expression of IgG2-mediated opsonic activity may be limited under conditions in which phagocytes with the R131 allotype of FcγRIIa are used as effectors in a bactericidal assay, as has been reported for other organisms (5). Accordingly, we are currently comparing the rates of IgG2-dependent killing of *A. actinomycetemcomitans* by neutrophils that express the R131 and H131 allotypes of FcγRIIa.

A key remaining question is whether LJP patients who produce substantial amounts of IgG2 antibodies directed to *A. actinomycetemcomitans* surface antigens have phagocytic cells that express the FcγRIIa allotype capable of effectively working with these antibodies. Many of the LJP subjects characterized by markedly elevated IgG antibody levels to *A. actinomycetemcomitans* are black (12, 19, 25, 36). The frequencies of expression of the H131 and R131 allotypes of FcγRIIa on the phagocytes of these subjects have not been determined. In this context, it has been noted that the frequencies of expression of the H131 and R131 allotypes can vary according to ethnic background (1, 21, 29). Thus, approximately 80% of black and

white subjects express the R131 allotype, in contrast to only 15% of Japanese subjects. It has been suggested that FcγRIIa allotype may be an important determinant of susceptibility to certain types of bacterial infection, particularly those situations in which IgG2 antibody response plays a key role in host defense (30). Consistent with this hypothesis, infections due to meningococci and encapsulated *Haemophilus influenzae* are less common in Japan than in the United States (10, 20). If black LJP subjects who produce large amounts of IgG2 antibodies to *A. actinomycetemcomitans* have phagocytes with the FcγRIIa allotype which does not recognize these antibodies (R131), such patients may be compromised with respect to clearance of this organism. In order to test this hypothesis, we are currently examining the distribution of FcγRIIa alleles in LJP subjects in general and in black LJP subjects in particular.

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