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Research Article

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Nonopsonic Antibodies in Cystic Fibrosis

Pseudomonas aeruginosa Lipopolysaccharide-specific Immunoglobulin G Antibodies from Infected Patient Sera Inhibit Neutrophil Oxidative Responses

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Abstract

Antibody opsonins from cystic fibrosis (CF) patients were investigated using nonmucoid and mucoid lipopolysaccharide (LPS) immunotype 1 Pseudomonas aeruginosa as bacterial ligands and PMN phagocytes. CF sera were compared to normal sera, polyvalent PA LPS hyperimmune globulin, and isotype switch variant monoclonal antibodies (MAbs) specific for type 1 PA LPS. Sera from PA-infected CF patients (CF PA+) had elevated levels of PA LPS and alginate IgG antibodies and promoted significantly greater antibody-dependent PMN chemiluminescence responses than sera from uninfected CF patients (CF PA−) or normal human sera (NHS). After adjustment for autologous IgG PA LPS antibody content, however, CF PA+ sera had less antibody-dependent opsonic activity than sera from CF PA− patients ($P < 0.025$) or NHS ($P < 0.0025$), suggesting qualitative opsonic defects of IgG PA LPS antibodies in CF PA+ sera. Antigen-specific immunoprecipitation of PA LPS antibodies enhanced opsonization by 40% of CF PA+ sera while uniformly reducing that from CF PA− sera ($P < 0.01$), indicating LPS-specific nonopsonic antibodies in some CF PA+ sera. Alginate antibodies were not critical opsonins in most uninfected CF patient sera. PA LPS IgG antibodies isolated by immunoaffinity chromatography from NHS, hyperimmune globulin, and CF PA− sources were opsonic and had greater activity at equal antigen-binding concentration than identical antibodies isolated from infected CF patients ($P < 0.01$–0.05); the majority of isolates from CF PA+ sera did not promote PMN oxidative responses above nonopsonic baseline. A potential isotypic basis for these findings was supported by differences in PMN responses to PA opsonized with MAbs of identical specificity but differing isotypes. PA LPS-specific IgG antibodies inhibiting PMN oxidative responses in infected patient sera demonstrate antigen-specific immunomodulation of host responses by chronic bacterial parasitism in CF, which may play a role in the pathophysiology of lung disease.

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Introduction

Cystic fibrosis (CF)[1] is characteristically complicated by chronic *Pseudomonas aeruginosa* (PA) endobronchial infection with progressive lung destruction (1). While the basis for increased susceptibility of CF patients to chronic PA lung infection remains unknown, notable defects in systemic immunity have not been found; it is therefore thought to be due to impaired expression of host defense mechanisms in the lung, such as altered respiratory epithelial adherence characteristics or mucociliary clearance (2).

Once the CF respiratory epithelium is colonized, it is very likely that additional factors come into play to allow persistence of PA endobronchial infection despite a vigorous immune response. These factors apparently include both adaptive changes in bacterial phenotype and maladaptive developments in host defense. With regard to the latter, perhaps the most striking observation has been that opsonization of PA with serum from CF patients infected with PA (CF PA+) reduces resulting lung macrophage phagocytosis and killing in vitro when compared with serum from healthy individuals or CF patients free of PA infection [CF PA−] (3–5). Notably, this opsonic defect is antigen-specific, as opsonization of other microbes with CF PA+ serum is normal (4–6). Defective opsonization has also been found using CF PA+ lipopolysaccharide (LPS)-specific IgG antibodies isolated from serum or bronchoalveolar lavage fluid, implicating these antibodies in the opsonic deficiency (7, 8). The defect has been demonstrated using lung macrophages from human and several other mammalian species, but was not apparent in one study when human PMN or monocytes were employed as target phagocytes in a single time point uptake assay (9). Discrepancies in phagocytosis of opsonized PA by different cell types may be due to heterogeneity in fine specificity and expression of IgG Fc receptors (FcγR) (10). For example, lung macrophages display a more restricted FcγR repertoire than PMN or monocytes as determined by IgG1–4 subclass binding and FcγR-mediated effector assays (11).

PA LPS IgG antibodies are partly fragmented in CF airways by proteolytic enzymes, potentially contributing to local opsonic deficiency (8). However, fragmentation is not found

1. *Abbreviations used in this paper:* BAL, bronchoalveolar lavage; CF PA+, cystic fibrosis patients infected with *P. aeruginosa;* CF PA−, cystic fibrosis patients without *P. aeruginosa* infection; CL, luminol-enhanced chemiluminescence; LPS, lipopolysaccharide; MPA, mucoid PA-1 strain CF/DL1; NGS, normal goat serum; NMPA, nonmucoid PA-1 strain ATCC 27312; PA-1, *P. aeruginosa* Fisher immunotype 1 LPS phenotype; Ps-ivIG, hyperimmune globulin enriched for PA LPS IgG antibodies.

in corresponding serum antibodies, which are also dysfunctional (3–5, 7, 12). The specificity of the opsonic defect suggests an antigen-driven Fcγ-based alteration in antibody function, such as production of functionally nonopsonizing isotypes (12, 13). This mechanism is also supported by induction of nonopsonizing serum antibodies in cats after parenteral hyperimmunization with PA LPS (14). Moreover, striking shifts in levels and proportions of IgG1-4 immunoglobulins, and more specifically in IgG1–4 antibody responses to PA LPS, occur in CF PA+ patients (12, 15–18).

In studying the CF opsonic defect, we revisited the question of whether it could be detected using PMN as target phagocytes. We reasoned that induction of nonopsonizing antibodies in CF PA+ sera could be detected with PMN if the magnitude, antigenic specificity, and isotypic distribution of antibody responses were taken into account. We therefore compared the opsonic activity of CF PA+ sera to that of CF PA− sera, normal nonimmune sera, polyclonal PA LPS antibody-enriched hyperimmune globulin derived from immune normal donors (Ps-ivIG), and a set of isotype switch murine monoclonal antibodies specific for PA LPS. IgG and IgG subclass antibody levels were determined and related to opsonic activity. The functional activity of isolated LPS antibodies, and the effect of their removal from serum, were assessed. We found that along with high levels of PA LPS-specific IgG, IgG2 and IgG4 antibodies, PA LPS-specific IgG antibodies reducing PMN oxidative responses can be induced by PA colonization. A potential isotypic basis for poor opsonization was supported by differences in PMN respiratory burst induction by switch variant monoclonal antibodies.

Methods

Bacteria. PA strains 27312–27318, representing the seven respective Fisher LPS immunotypes, were obtained from the American Type Culture Collection, Bethesda, MD. Immunotype 1 LPS (PA-1) strain 27312 was routinely employed as the nonmucoid test organism (NMPA), with immunotype 4 ATCC 27315 as the nonmucoid LPS control strain. Mucoid phenotype PA strains were isolated from sputum cultures of CF patients attending the Stanford CF Center Clinic by the Children's Hospital-Stanford clinical microbiology laboratory. Mucoid strain CF/DL1 was selected as the mucoid test organism (MPA) on the basis of three criteria: (*a*) resistance to complement-mediated bactericidal activity at serum concentrations of up to 50%, (*b*) relatively low nonopsonic PMN phagocytosis, and (*c*) strongly positive agglutination by Fisher immunotype 1 antisera. Mucoid stability was rechecked by visual inspection of periodic subcultures from stored frozen isolates. Bacteria were isolated from stock tryptic soy broth and stored in glycerol at −20°C. Subcultures were thawed and grown up overnight in tryptic soy broth at 37°C. They were sedimented by centrifugation at 2,500 rpm for 10 min at 4°C, washed three times in PBS (Gibco Laboratories, Grand Island, NY) 0.1% gelatin and resuspended in HBSS (Gibco). The suspension was adjusted spectrophotometrically to 2.5×10^7 cfu/ml. Adjusted bacterial suspensions were kept refrigerated until use. PA strains were periodically serotyped using standard IATS slide agglutination typing sera according to the manufacturer's instructions (Difco Laboratories, Detroit, MI).

Opsonins. Venous blood samples were allowed to clot for 60 min at room temperature, decanted after centrifugation, and aliquots were frozen at −70°C. Serum samples were obtained from 20 CF PA+ patients, ages 9–38 yr (mean 21.9±6.5 yr) and 14 CF PA− patients, ages 14–30 yr (mean 22.7±5.4). Each sample was tested individually in the assays described below. CF PA+ patients were defined by the dual criteria of chronic sputum colonization and elevated serum IgG antibodies to polyvalent PA LPS (Pseudogen; Parke-Davis Co., Detroit, MI) (19). For the present study, PA LPS immunotype 1 IgG antibody levels were also determined to establish immunogenic exposure. All CF PA+ subjects had elevated IgG titers to this antigen; in addition, 1 CF subject with no PA in sputum cultures had an elevated serum antibody level but was classified as CF PA− on the microbiologic criterion. This case may represent an example of early colonization undetected by sputum culture (20). Sera from 10 healthy adult laboratory personnel with no history of PA infection were initially tested individually and later pooled for use as normal human serum (NHS) controls. Antibody-mediated opsonization was differentiated from complement-induced or supported effects by heat inactivation of sera at 56°C for 30 min. In opsonic experiments, 1% exogenous whole human complement (Cordis Laboratories, Miami FL) was added to heat-inactivated sera and tested along with the corresponding native and decomplemented samples.

Hyperimmune globulin derived from selected normal adult volunteer plasma screened for high titers of antibodies against PA LPS immunotypes 1, 2, 4, or 6 (Ps-ivIG) was provided by Dr. Michael Collins of Cutter Laboratories, Berkeley, CA (21). Three PA-1 specific isotype switch variant murine monoclonal antibodies (IgM isotype MAb clone 11.12, IgG2a MAb clone 16.17, and IgG2b MAb clone 19.63) were provided in the form of clarified hybridoma ascites fluid by Dr. James Larrick of Cetus Immune Research Laboratories, Palo Alto, CA (22). Immunotype specificity was demonstrated by LPS- and bacterial-binding ELISA (23). Immunoblots showed that these MAbs bind epitopes on PA-1 high molecular weight *o*-polysaccharide side chains without binding to LPS of other PA immunotypes, other Pseudomonas species, or to other gram-negative LPS antigens (unpublished data).

Serum sensitivity. Susceptibility of PA strains to the bactericidal ec activity of cell-free fresh normal human serum (NHS) was assayed by suspension of stock culture samples placed in Trypticase soy broth at an optical density of 0.1 at 650 nm to an endpoint optical density of 0.4, following by centrifugation and resuspension in an equal volume of minimal essential medium (MEM), dilution to 10^{-3} and 10^{-4} in MEM, and addition of 0.1 ml bacterial suspension to 0.1 ml NHS. After incubation for 1 h at 37°C, bacteria were plated on soy agar (24). Control tubes contained MEM instead of NHS. Strains sensitive to 50% NHS were also tested with 5 and 1% NHS.

Isolation of PMNs. Anticoagulated (EDTA 3% wt/vol) whole blood was obtained from healthy volunteers, immediately layered on Ficoll-Hypaque (Mono-Poly resolving medium; Flow Laboratories, McLean, VA) and centrifuged at 300 *g* until two distinct leukocyte bands were visible (25). The PMN layer was carefully pipetted off, washed once in HBSS-0.1% gelatin and adjusted by hemocytometer to 5×10^5 PMN/ml in HBSS. By this method 97% of the cells obtained were PMN, of which > 95% were viable by trypan blue dye exclusion.

Opsonization. Bacteria were mixed with serum at a ratio of four parts bacterial suspension to one part 10% serum (Ps-ivIG was used at 5%) for 30 min at 37°C under continuous shaking. Preliminary dose-response experiments using final opsonin concentrations of 0.1–20% showed that these opsonin concentrations (2% for sera, 1% for Ps-ivIG) provided adequate opsonization as measured by PMN chemiluminescence response. Opsonization was stopped with ice cold PBS-0.1% gelatin. The bacterial suspension was then washed twice with PBS-0.1% gelatin, resuspended to its original volume in HBSS (yielding a bacterial density of 2.5×10^7 cfu/ml) and immediately employed.

PMN chemiluminescence (CL). Luminol-amplified PMN CL was measured at 37°C using opsonized PA. Ps-ivIG was included in each experiment as a positive control and unopsonized PA was used as a baseline "negative" control. In experiments with MAbs, an irrelevant murine IgG1 antihuman IgE MAb was also included as a negative control. PMN CL was measured in an automated luminometer (LKB 1251; Wallac Oy, Turku, Finland) connected to a video terminal and printer, allowing continuous registration via digital printout. The light output was recorded in millivolts and updated each second. The microprocessor calculated the integral (mV-s) representing the total

amount of light emitted during a selected time interval. Samples were run at 37°C under continuous shaking. The CL reaction mixture consisted of 500 μl PMN (5×10^5 cells/ml), 400 μl HBSS containing 10^{-6} M luminol (5-amino-2,3-dihydro-1,4-phtalazinedione; LKB) and 100 μl opsonized bacterial suspension, resulting in a 1.0-ml final volume with a PA:PMN ratio of 10:1. Final PA:PMN ratios were monitored by quantitative cultures of the final mixtures at zero time. Where unexpected bacteria loss occurred (e.g., by adherence to tube surfaces) bacterial inoculum was increased to maintain ratios ≥ 10:1, as ratios below this did not result in satisfactory CL signals.

After equilibration in the luminometer counting chambers for at least 1 h at 37°C, unstimulated PMNs were monitored for return of CL to background emission levels (≤ 0.5 mV). Then, immediately after the addition of the bacterial suspension, light emission of each vial was recorded for 6 s and at subsequent 5-min intervals for 150 min. All determinations were performed in duplicate. Results were expressed as the mean value after automatic subtraction of background.

Bactericidal assay. PMN were isolated as described above, adjusted to a final concentration of 10^6 cfu/ml, and incubated with 10^7 cfu/ml PA in duplicate tubes at 37°C for 5–90 min. Phagocytosis was stopped by addition of cold PBS. PMN-associated PA were isolated by centrifugation at 160 *g* for 5 min at 4°C. Bactericidal activity was determined by digestion of PA-associated PMN pellets with 1 N NaOH or 1% Triton for 10 min under vigorous vortexing. Serial 10-fold dilutions of samples in PBS were prepared, and 1-ml aliquots plated on MacConkey media plates. Colony forming units were counted after 1–2 d incubation at 37°C.

PA LPS and alginate-specific IgG serum antibodies. Samples were tested for IgG antibodies to PA-1 LPS using purified antigen isolated from the autologous ATCC strain 27312 (List Biological Laboratories, Campbell, CA). Serum PA-1 LPS IgG antibody content was measured by ELISA as previously described, with an internal intraplate high-titer standard serum whose ELISA unit values (EU) were calibrated by an anti-isotype standardization method, where 1 EU corresponds to ≈ 0.01 μg/ml (19).

Alginate antibodies were determined using commercial sodium alginate (Sigma Chemical Co., St Louis, MO) as the antigen (26–28). Alginate 200 μg/ml in carbonate buffer pH 9.6 was incubated in polyvinylchloride flat bottom microtiter plate wells (100 μl/well) 1 h at 37°C. After aspiration, 1% normal goat serum (NGS) in PBS was added (200 μl/well), incubated 1 h at 37°C, aspirated, and wells washed three times with PBS-0.1% Tween. Serum samples diluted in NGS-PBS were added (100 μl/well) for 2 h at 37°C, aspirated, and wells washed. Polyclonal goat antihuman IgG-peroxidase conjugate (Sigma Chemical Co.) 1:1,000 in NGS-PBS was then added, incubated 1 h at 37°C, aspirated, and wells washed. The reaction was developed with *o*-phenylene-diamine 0.5 mg/ml in citrate-phosphate buffer, pH 6.0, containing hydrogen peroxide (4 μl 30% H_2O_2/10 ml substrate buffer) for 15 min at room temperature. The reaction was stopped with 5 N H_2SO_4 and absorbance read at 492 nm in a microplate spectrophotometer. Standard curves were constructed with a single intraplate high-titer serum sample.

Logit curves were constructed with a micro-ELISA software package (MacReader 2.0; Bio-Rad Laboratories, Richmond CA). The mean of duplicate test sample results were interpolated to the linear region of the standard curve and expressed in ELISA units (EU). Serum PA LPS and alginate antibody results in respective EU are not quantifiable with reference to the other antigen.

ELISA specificity. The specificities of the PA LPS and alginate ELISA for their respective antigens were tested as follows. Screened CF PA+ serum standards containing both high titer IgG LPS and alginate antibodies, and a low titer normal control sample, were each serially diluted 10-fold (neat to 10^{-4}) and incubated in microfuge tubes in checkerboard fashion with an equal volume of each antigen discretely at three log concentrations (1 mg, 100 μg, and 1 μg/ml for alginate; 10 mg, 1 mg, and 100 μg/ml for PA LPS). After incubation for 3 h at 22°C followed by overnight incubation at 4°C, the mixtures were then centrifuged at 3,000 rpm for 20 min. The supernatants were then tested in ELISA along with corresponding untreated samples. Percent inhibition by preincubation with autologous and heterologous antigen was calculated.

Postantigen absorption CL studies. To determine the effect of removal of PA LPS or alginate antibodies on PMN CL responses, serum samples were incubated with the respective antigens in a checkerboard fashion. Serial dilutions of sera were incubated with LPS and alginate essentially as described above for ELISA specificity studies. Sera diluted in HBSS-Ca^{2+} were first decomplemented by heat inactivation at 56°C for 30 min, followed by incubation with dilutions of antigen in identical buffer overnight at 4°C. For most samples, immunoadsorption was maximal at 1:20 serum dilution with 500 μg/ml LPS concentration. Samples were then centrifuged at 2,500 rpm for 90 min and the supernatant decanted for postadsorption assay. Specific immunoprecipitation of autologous antibody was verified by ELISA. The sample for each individual demonstrating maximal immunoprecipitation was concentrated to initial volume by pervaporation and subsequently used to opsonize PA for simultaneous PMN CL assay along with the corresponding unadsorbed autologous serum sample.

Isolation of PA-1 LPS antibodies from serum. PA-1 specific IgG antibodies were isolated from serum essentially as described by Fick et al. (29). PA-1 immunoadsorbent columns were prepared as follows: 6 g cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals) was washed with 1,200 ml 0.001 M HCl on sintered glass filters and then transferred to 50-ml conical plastic tubes containing 30 ml coupling solution (0.1 M $NaHCO_3$–0.5 M NaCl, pH 8.0) and 50 mg PA-1 LPS (List Biological Laboratories). After gentle shaking at room temperature for 2 h and incubation overnight at 4°C, the gel was washed with 500 ml coupling solution on a sintered glass filter. Remaining open sites were blocked by incubation with 1 M ethanolamine, pH 9.0, for 2 h. The gel was then alternately washed with coupling solution and acetate buffer (0.1 M acetate-0.5 M NaCl pH 4.0). The gel was stored in borate buffer (0.2 M H_3BO_3-0.5 M NaCl, pH 8.0). Elution of bound antibodies was performed with 0.05 M glycine HCl, pH 2.4. Control columns were prepared in the same fashion using 50 mg BSA (Sigma Chemical Co.) instead of PA LPS.

Serum IgG fractions were prepared by ammonium sulfate precipitation and ion exchange chromatography. Serum samples were mixed with half volumes (33%) of saturated $(NH_4)_2SO_4$ solution for 2 h at room temperature. After centrifugation for 20 min at 13,000 *g* at 4°C, precipitates were dissolved in distilled water and dialyzed extensively against 0.01 M phosphate buffer, pH 8.0. After repeat centrifugation supernatants were used for DE-52 (diethylamino-ethylcellulose; Whatman, Inc., Clifton, NJ) ion-exchange column chromatography. Columns were equilibrated with 0.01 M phosphate buffer, pH 8.0. Approximately 3.5-ml vol of supernatant was applied to the DE-52 column and 5-min timed fractions were collected and assayed for protein at OD_{280} nm. Protein peaks were assayed for immunoglobulins by double immunodiffusion against antisera to human IgG, IgM, and IgA. The major, early IgG-containing portion of the first peak was dialyzed extensively in borate-saline buffer, pooled, assayed for protein at OD_{280}, and used for the antibody isolation procedure.

Antibody isolations from IgG serum fractions were performed by pipetting off borate buffer from immunosorbent gels, leaving ~ 10 ml gel volume. Then 5–10 ml (1–4 mg/ml) IgG fraction was added and gently mixed at room temperature for 20 min and passed through a sintered glass filter, followed by a second incubation and filtration. The gel was then extensively washed (150 ml) with borate buffer until the OD_{280} of the wash eluate was < 0.02. Antibodies were then eluted from the gel with 10 ml glycine HCl passing through the filter twice. The eluate was transferred into a tube with 1 ml 5% NH_4HCO_3 to keep the pH close to 7.0. The solution was then dialyzed with saline and concentrated down to 0.5–1 ml by centrifugation at 3,000 *g* using Centriprep 10 concentrators (Amicon Corp., Danvers, MA). The gel was regenerated by washing with 100 ml glycine buffer followed by three 50-ml washes with borate-saline buffer. The protein level of collected fractions was determined spectrophotometrically at OD_{280} and the PA-1 antigen-binding activity by ELISA. For functional studies, PA-1

antibody isolates were employed both at maximal concentration and again after dilution to equal antigen-binding titers as determined by ELISA. PA-1 was opsonized with antibody isolates as described above.

PA LPS and alginate IgG subclass antibodies. Subclass-specific ELISA for IgG2 and IgG4 antibodies to PA-1 LPS and alginate were performed as previously described (12, 13). The only modification involved use of mixtures of IgG subclass-specific anti-isotypic detector MAbs. In the IgG2 assay, a 1:1 mixture of clones GOM-1 and HP6014 (ICN, Costa Mesa, CA) were used at dilutions of 1:2,000. In the IgG4 assay, 1:1:1 mixes of clones SK-44 (ICN), RJ4 (Oxoid), and 186 (WHO Collaborating Center for Immunoglobulin Subclasses, Centers for Disease Control, Atlanta, GA) were used at dilutions of 1:4,000. Reactions were developed with affinity-purified goat antimouse IgG peroxidase conjugate (Boehringer-Mannheim, Indianapolis, IN) at 1:1,000 dilution with *o*-phenylenediamine substrate as previously described.

Statistical analysis. Paired or unpaired Student's *t* tests were used to test for significance of differences of mean CL values within or between subgroups, respectively. Bonferroni corrections were made for multiple comparisons where applicable. *P*-values reported are for two-tailed tests, with P values of < 0.05 considered significant. Antibody values were logarithmically transformed prior to statistical analysis. Linear correlation coefficients were determined by the least squares method and Spearman rank order correlations determined where appropriate. Statistical operations were performed using the Statview 512+ software package (Brain Power Inc., Calabasas, CA). Mean values are given along with standard errors and 95% confidence intervals where appropriate.

Results

Opsonization of nonmucoid PA. The addition of unopsonized NMPA to temperature-equilibrated normal human PMN yielded low chemiluminescence responses (peak CL = 5.4±1.2 mV-s). PMN CL responses to opsonized NMPA, shown in Fig. 1, significantly increased after opsonization with CF PA+ sera (mean peak CL = 26.3±2.9, $P < 0.005$) and, to a lesser degree, with CF PA− sera (mean peak CL = 11.7±1.7, $P < 0.05$). Induction of PMN oxidative activity by CF PA+ sera was not significantly different in kinetic pattern or peak responses than that obtained with Ps-ivIG. In contrast, PMN CL activity induced by PA opsonized with CF PA− sera was significantly lower than Ps-ivIG or CF PA+ sera and similar to that promoted by NHS (Fig. 1). Heat inactivation of opsonic sources at 56°C for 30 min did not significantly diminish PMN CL-producing activity (not shown), indicating that this NMPA strain is opsonized for promotion of PMN oxidative metabolism by natural human antibodies in the absence of complement.

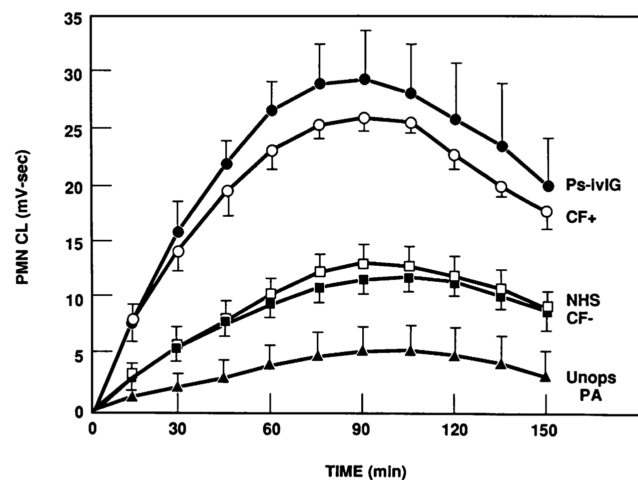

Figure 1. PMN chemiluminescence (CL) responses (mean±SE) to opsonized nonmucoid PA-1 strain ATCC 27312. Peak PMN CL-inducing opsonic activity of Ps-ivIG and CF PA+ sera (n = 20) was significantly greater than CF PA− sera (n = 14) and NHS (n = 10) ($P < 0.005$ for each). Ps-ivIG and CF PA+ sera were similar in PMN CL-inducing activity, as were CF PA− sera and NHS. Unopsonized PA-1 induced less PMN CL than PA-1 opsonized with Ps-ivIG or CF PA+ sera ($P < 0.005$ for each), or CF PA− sera or NHS ($P < 0.05$ for each).

Opsonization of mucoid PA. Among several CF-derived mucoid PA strains we examined, DL1 was selected on the basis of three criteria. First, this strain was resistant to serum bactericidal activity at serum concentrations up to 50%, and therefore suitable for use in opsonic assays. Second, although it polyagglutinated with IATS standard typing sera, the strongest agglutination occurred with IATS type 6 antiserum (Fisher immunotype 1). There was weak agglutination with IATS types 4, 9, and 10 antisera and trace agglutination with types 1, 2, and 3 antisera. Although we did not directly assess immunotype 1 side chain production, opsonization of MPA by PA LPS immunotype 1-specific MAbs (see below) confirmed the surface expression and accessibility of PA-1 epitopes. Therefore, DL1 was assumed to express both alginate and LPS immunotype 1 antigens amenable to ELISA analysis. Third, PMN oxidative and bactericidal responses to unopsonized DL1 were low, allowing assessment of opsonin-induced changes.

As shown in Fig. 2, opsonization of MPA with CF PA+ sera produced PMN CL responses which exceeded those of either Ps-ivIG or NHS. Heat inactivation of complement present in CF PA+ sera did not reduce opsonization, suggesting that immune antibodies were responsible for the CL responses. Peak PMN CL responses to MPA opsonized with Ps-ivIG occurred earlier than those to MPA opsonized with NHS. Opsonization of MPA by pooled NHS was dependent on both complement and antibody, since peak PMN CL response (35 mV-s) was diminished by heat inactivation (25 mV-s) and augmented by subsequent add-back of exogenous complement (57 mV-s). Peak PMN CL response to MPA opsonized by Ps-ivIG (52 mV-s) was slightly reduced by similar heat treat-

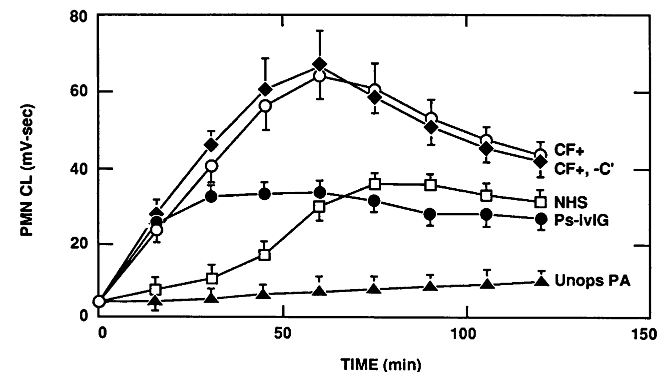

Figure 2. PMN CL responses (mean±SE) to opsonized mucoid PA-1 strain CF/DL1. Opsonization of DL1 is shown for native CF PA+ sera (n = 20) and after decomplementation by heat inactivation (−C′). CF PA+ sera induced higher peak CL responses than other opsonic sources ($P < 0.01$).

ment (48 mV-s) and augmented by addition of complement (75 mV-s). Complement repletion produced earlier peak CL responses with pooled NHS but later peak CL responses with Ps-ivIG. The differential effect of complement repletion on kinetics could be related to the different properties of native serum and Ps-ivIG, which is > 98% IgG stabilized in 10% maltose.

Isotype-dependent opsonization. The relationship between isotype and opsonization was examined by comparing PMN CL-inducing activity of NMPA and MPA after opsonization by isotype switch variant PA type 1 LPS-specific MAbs. Preliminary dose-response experiments showed parallel titration slopes in the ELISA and PMN CL assays for the three MAbs, indicating similar antigen affinities. Prozone inhibition occurred at MAb protein concentration ≥ 100 μg/ml for each of the clones. MAbs were used to opsonize bacteria at equal antigen-binding titers. Opsonizing activity was observed over a concentration range of ~ 100 ng–100 μg/ml. Antigen specificity was confirmed using an immunotype 4 NMPA strain (PA-4). Opsonization of PA-4 was not augmented by any of these PA-1 specific MAbs in the absence of complement. Opsonization of PA-4 by the MAbs in the presence of complement was enhanced by the three MAbs but to no greater degree than that obtained by opsonization with complement alone or by a negative control MAb.

Fig. 3 depicts PMN CL responses induced by NMPA after opsonization by each of the three MAbs in the presence of complement. At equal antigen-binding concentrations (ascites dilutions 1–2%, protein concentration ~ 10 μg/ml) all three MAbs promoted PMN respiratory burst activity with a rank peak PMN CL induction order of IgG2b > IgG2a > IgM. The IgG MAbs induced earlier as well as greater peak CL responses than the IgM MAb.

These experiments were repeated employing the MPA. Resulting PMN CL responses induced by the three MAbs in the presence of complement are shown in Fig. 4. The same rank order activity, IgG2b > IgG2a > IgM, was found with MPA as for NMPA, although peak PMN responses were somewhat higher and occurred slightly earlier with MPA than NMPA. PMN bactericidal assay results were concordant with CL responses. MPA was efficiently killed after opsonization by all three MAbs in the presence of complement (log reduction

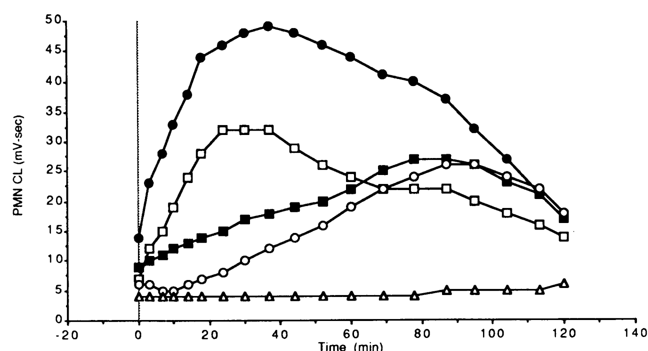

Figure 3. PMN chemiluminescence responses to nonmucoid PA-1 opsonized with PA-1 LPS specific monoclonal antibodies in the presence of 1% human complement. MAb concentrations were adjusted to opsonize PA-1 at equal PA-1 LPS antigen-binding titers. •, IgG2b MAb 19.63; ○, control MAb; □, IgG2a MAb 16.17; ■, IgM MAb 11.12; △, no opsonization.

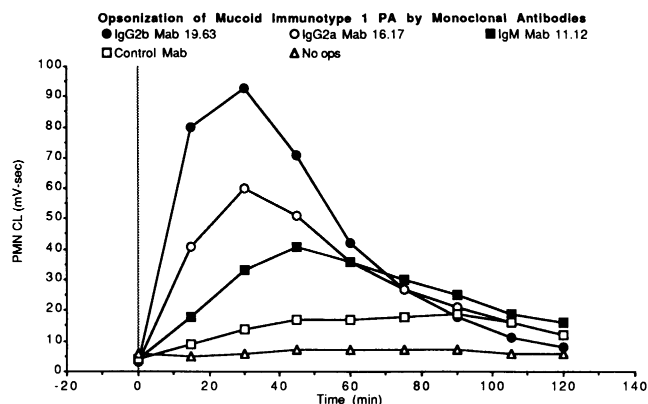

Figure 4. PMN chemiluminescence responses to mucoid PA-1 opsonized with PA-1 LPS specific monoclonal antibodies in the presence of complement. Conditions were identical to those illustrated in Fig. 3 for nonmucoid PA-1. •, IgG2b MAb 19.63; ○, IgG2a MAb 16.17; □, control MAb; ■, IgM MAb 11.12; △, no opsonization.

> 2 at 30 min). Killing of MPA in the absence of complement was greatest after opsonization with the IgG2a or IgG2b MAbs, followed by the IgM MAb (log reductions of cfu/cm^3 at 30 min = 1.90, 1.85, and 1.72, respectively; nonopsonic killing = 1.01 log reduction).

Serum PA antibodies and opsonization. PA LPS and alginate IgG, IgG2, and IgG4 antibody responses were evaluated by enzyme-linked immunosorbent assays. Assay specificity was examined by cross-inhibition experiments in which various concentrations of each antigen were preincubated with heat-inactivated sera and resultant complexes removed by immunoprecipitation before ELISA (see Methods). Inhibition of antibody binding to immobilized PA LPS in a high-titer standard serum was dose-dependent and antigen-specific: at 10^{-2} serum dilution, LPS antibody binding was inhibited 40% by preincubation with 100 μg/ml LPS, 88% with 1 mg/ml, and 95% by 10 mg/ml, while no inhibition occurred after preincubation with alginate over a 4 log range (1–1,000 μg/ml). Conversely, antibody binding to alginate was inhibited 66% by 1 μg/ml alginate, 72% by 100 μg/ml, and 83% by 1 mg/ml, while LPS inhibited by only 9–19% at 0.1–10 mg/ml.

Serum IgG, IgG2, and IgG4 PA LPS antibody levels in three study groups are shown in Table I. CF PA+ serum anti-

Table I. Serum Pseudomonas aeruginosa Immunotype 1 LPS Geometric Mean Antibody Levels in CF Patient Groups and Controls

Isotype	CF PA+	CF PA−	NHS
IgG, EU	7406*	202	184
(range)	(280–56,800)	(68–1,664)	
IgG2	1548*	61	185
	(75–35,600)	(2–740)	
IgG4	869*	100	51
	(60–4,735)	(8–1,020)	

LPS, lipopolysaccharide; CF PA+, patients with cystic fibrosis infected with PA; CF PA−, CF patients not infected with PA; NHS, normal human serum; EU, ELISA units
* $P < 0.001$: CF PA+ vs. CF PA− or NHS.

body levels were higher compared to CF PA− sera or NHS ($P < 0.001$), which were not significantly different from each other. PA LPS IgG antibody levels for all study subjects analyzed together correlated with autologous peak PMN CL responses to opsonized NMPA ($r = 0.46$, $P < 0.01$). However, this correlation appeared attributable to the contribution of NHS and CF PA− opsonins because PA LPS antibody levels in the CF PA+ group analyzed separately did not correlate with PMN CL responses ($r = 0.16$).

The peak PMN CL response induced by opsonization of NMPA with heat-inactivated serum for each individual was divided by the autologous PA LPS IgG antibody concentration in order to derive a value for antibody-mediated opsonization at equivalent antigen-binding level. Fig. 5 displays the PMN CL-producing activity of CF PA+ sera, CF PA− sera, and NHS after this adjustment. Viewed in this way, CF PA+ sera had significantly lower opsonic activity than CF PA− sera ($P < 0.025$) or NHS ($P < 0.0025$), suggesting a qualitative opsonic defect in CF PA+ LPS IgG antibodies.

CF PA+ patients had highly elevated levels of alginate-specific IgG antibodies when compared with CF PA− patients or NHS ($P < 0.005$), who had similar levels of these antibodies (Table II). Among CF patients, there was a positive correlation between levels of LPS and alginate IgG antibodies ($r_s = 0.65$, $P < 0.001$). CF PA+ patients also had elevated levels of alginate-specific IgG2 subclass antibodies compared to CF PA− or NHS ($P < 0.05$). In contrast to antibody responses to LPS, IgG4 antibodies to alginate were detectable in only a few CF PA+ sera at low levels, suggesting lesser immunogenicity and possibly greater isotypic restriction to this antigen. There was a strong correlation between IgG and IgG2 antibody responses to alginate in CF patients ($r_s = 0.78$, $P < 0.0001$).

To determine whether LPS or alginate antibodies were more closely associated with opsonization, correlation coefficients were determined comparing IgG antibody levels in CF sera to peak PMN CL responses induced by opsonization of MPA with heat-inactivated sera. A correlation was found between IgG LPS antibody levels and CL responses ($r_s = 0.44$, $P = 0.01$) but not for alginate ($r_s = 0.07$), suggesting that opsonization of MPA may be mediated by LPS antibodies in CF sera. This would be consistent with PMN CL induction by LPS-producing MPA opsonized with Ps-ivIG and LPS-1 MAbs (Figs. 2 and 4).

PA antibody specificities and opsonic responses in uncolonized CF patients. CF PA− serum samples were found to display a heterogeneity of opsonic activity in PMN CL assay, which fell into two quite different patterns shown in Fig. 6. 5 of 14 CF PA− sera promoted a highly opsonic PMN CL response

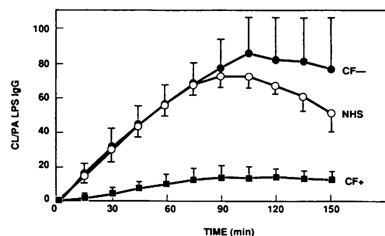

Figure 5. PMN chemiluminescence responses to nonmucoid PA-1 opsonized by CF PA+, CF PA−, or normal sera. The results have been corrected for autologous PA-1 LPS IgG antibody content. Opsonization of PA-1 was reduced in CF PA+ compared to CF PA− or normal sera ($P < 0.01$ for all time points past baseline). The differences between CF PA− and NHS at 90–150 min were not statistically significant.

Table II. Serum Alginate Geometric Mean Antibody Levels in CF Patient Groups and Controls

Isotype	CF PA+	CF PA−	NHS
IgG, EU	6540*	572	206
(range)	(70–90,000)	(33–1,900)	(10–3,000)
IgG2	1195‡	607	416
	(220–9,600)	(100–3,840)	(100–1,160)

* $P < 0.005$: CF+ vs. CF− or NHS.
‡ $P < 0.05$: CF+ vs. CF− or NHS.

similar to that seen with CF PA+ sera, despite low ELISA levels, comparable to NHS, of LPS and alginate antibodies. This opsonization was not due to complement because it was not reduced by heat inactivation (Fig. 6 *A*). However, most CF PA− sera induced PMN CL responses similar to those induced by NHS (Fig. 6 *B*). Thus, 36% of CF PA− sera differed from NHS in the apparent presence of heat-stable factors that opsonized MPA without complement. Adjustment of CL responses for autologous alginate antibody levels did not suggest qualitative differences between CF PA+ and NHS as found with LPS antibodies.

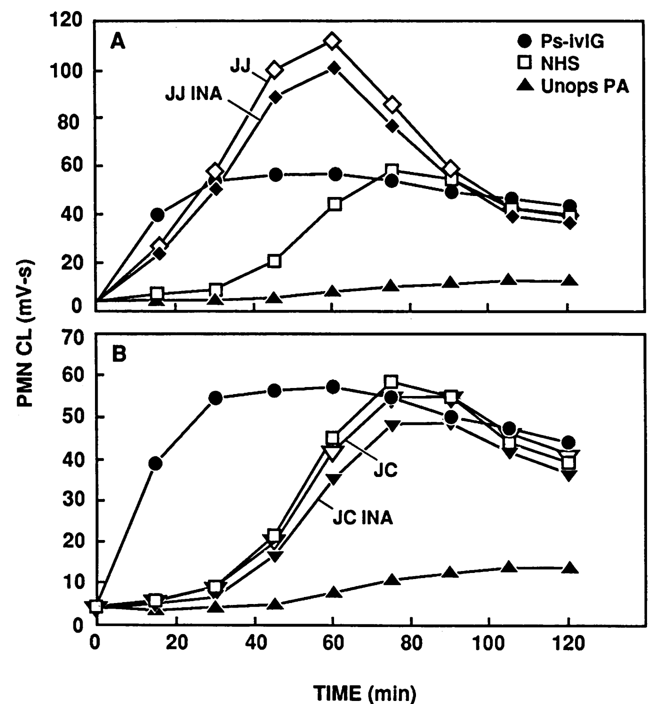

Figure 6. (*A*) PMN chemiluminesence response to mucoid PA-1 strain CF/DL1 opsonized by native serum of CF PA− patient J.J. and after heat inactivation of complement. This PMN CL curve was representative of responses induced by 5/14 of the CF PA− sera. This subgroup quantitatively and kinetically resembled the CF PA+ sera, despite great disparity in PA-1 LPS and alginate antibody levels in the two groups. (*B*) Opsonization of DL1 by serum of CF PA− patient J.C., which was representative of PMN CL responses induced by a majority (9/14) of the CF PA− sera. This pattern of opsonization of mucoid PA-1 resembled that seen with NHS, showing lower and later peak PMN CL induction than CF PA+ sera or Ps-ivIG.

Nonopsonic antibodies in CF sera. The results seen in PMN CL assays after correcting for autologous IgG antibody levels (Fig. 5) indirectly suggested that some LPS antibodies might be nonopsonizing in CF PA+ patients. Therefore, sera from 10 CF PA+ and 9 CF PA− patients were subjected to immunoadsorption with PA LPS and alginate. The adsorbed samples were then tested alongside native sera in PMN CL assay. As shown in Fig. 7, removal of PA LPS antibodies from CF PA+ sera enhanced PMN CL responses in 4 of 10 patient samples and had no effect in two others. In contrast, adsorption of LPS antibodies from CF PA− sera uniformly inhibited PMN CL responses (mean net change, 51% inhibition; difference in group means significant at $P < 0.01$, Mann Whitney U test). Adsorption of alginate antibodies slightly decreased PMN CL mean responses in both groups (net change 27 vs. 7% inhibition in CF PA+ and CF PA−, respectively, ns). Interestingly, three CF PA− sera showed alginate blocking activity with 33–120% increase in PMN CL after alginate adsorption.

Opsonic function of isolated PA-1 LPS IgG serum antibodies. To further assess the specificity and isotypic basis of diminished opsonic function of LPS antibodies in CF PA+ sera, IgG antibodies to PA LPS were isolated from sera by sequential ammonium sulfate precipitation, ion exchange chromatography, and immunoaffinity chromatography. BSA was used as a control for nonspecific adsorption of antibodies to activated Sepharose; separate aliquots of each processed serum sample were affinity purified on PA LPS and BSA, respectively.

PA LPS antibodies were isolated from sera of 11 CF PA+ sera and compared to those isolated from 12 CF PA− sera, pooled NHS, and Ps-ivIG. PA LPS antibody ELISA levels were determined on IgG fractions isolated from sera before and after LPS antibody affinity purification. The specificity of the purification was confirmed by high antibody levels from CF PA+ and Ps-ivIG fractions eluted off the LPS affinity column in comparison to that eluted off the BSA column (mean net OD_{490} of undiluted eluate = 0.53±0.08 for LPS eluate vs. 0.09±0.03 for BSA eluate, $P < 0.001$). ELISA antibody levels in the isolates from the CF PA+ group were as expected severalfold higher than those in the CF PA− group (195±56 vs. 42±26 ELISA units, $P < 0.025$); Ps-ivIG level was 106 EU (not significantly different from CF PA+, one-sample t test) and PNHS level was 7 EU (not significantly different from CF PA−).

Isolated PA LPS IgG antibodies were adjusted to identical ELISA antigen-binding concentrations before opsonizing NMPA with complement in PMN CL assay. As shown in Fig. 8, peak 90-min PMN CL induction was equivalent for PA LPS IgG antibodies isolated from Ps-ivIG (mean of seven separate opsonizations = 11.21±0.94 mV-s, 95% CI = 8.9–13.5), pooled NHS (mean of four opsonizations = 13.37±1.3, 95% CI = 9.1–17.6), and CF PA− (9.96±1.01, 95% CI = 7.7–12.2). Ps-ivIG, pooled NHS, and CF PA− serum derived antibodies all showed positive opsonic effect compared to PMN CL responses to unopsonized NMPA ($P < 0.01$–0.001).

In contrast, PMN CL induction by the isolated PA LPS IgG antibodies from CF PA+ sera (6.57±0.98, 95% CI = 4.4–8.8) was significantly lower than those from CF PA− ($P < 0.05$, two-tailed t test), Ps-ivIG ($P < 0.01$), or pooled NHS ($P < 0.01$). Dilution of antibody concentrations present in native samples to achieve equal antigen-binding concentrations for opsonization could not account for the reduced opsonization seen with CF PA+ sera because dilution of Ps-ivIG was as great as any CF PA+ serum sample, and several of the latter were diluted to a lesser extent but nevertheless had reduced activity. Seven of 11 CF PA+ samples showed PMN CL-induction activity equivalent to or less than that produced by unopsonized NMPA alone ($n = 6$, 5.02±0.28, 95% CI = 4.3–5.7). In contrast, lack of opsonization was found in only 2 of 12 CF PA− serum-derived antibodies (chi-square = 5.3, $P = 0.02$). Viewed as percent change from unopsonized CL response, Ps-ivIG, pooled NHS, and CF PA− LPS antibodies produced

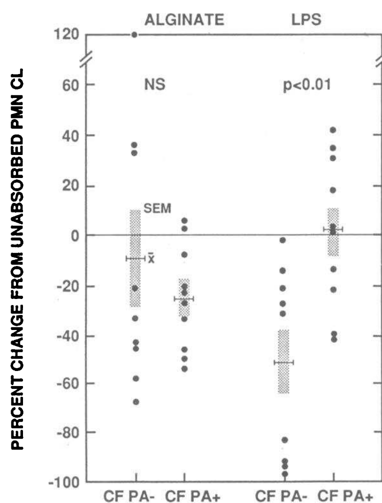

Figure 7. Preincubation of sera from CF PA+ patients with PA LPS to remove PA LPS antibodies resulted in enhanced PMN CL induction by mucoid PA-1 opsonized by the adsorbed sera in 4/10 patients and no effect in 2 others when results were compared to simultaneous PMN CL induced by autologous native unadsorbed sera. (The overall mean change for the group was +2%.) PA LPS antibody adsorption from PA CF− patient sera ($n = 9$) uniformly inhibited PMN CL responses, with a mean decrease of 51%. The difference between the two groups was significant ($P < 0.01$, Mann Whitney U test). Adsorption of alginate-binding antibodies modestly decreased PMN CL induction in both groups of CF patients (mean inhibition, 27% in CF PA+ compared to 9% in CF PA−, not significant). The group means±SD are also indicated.

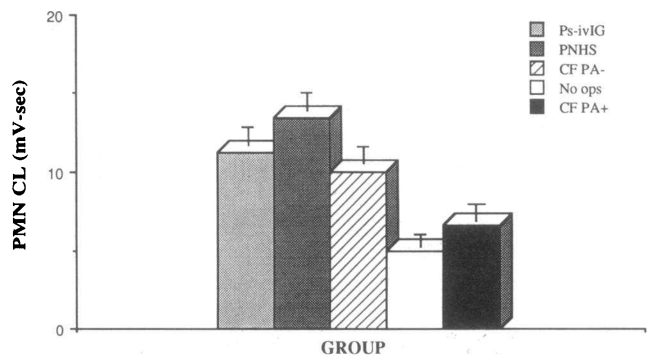

Figure 8. Peak PMN chemiluminescence induction by nonmucoid PA-1 opsonized with equal antigen-binding ELISA levels of IgG PA-1 LPS antibodies isolated from serum or hyperimmune globulin [Ps-ivIG] with complement. Peak PMN responses were significantly lower to PA-1 opsonized with CF PA+ derived antibodies than PA-1 opsonized with antibodies isolated from Ps-ivIG ($P < 0.01$), pooled normal human serum (NHS) ($P < 0.01$), or CF PA− sera ($P < 0.05$). PMN responses to PA opsonized by Ps-ivIG, pooled NHS, and CF PA− antibodies were all greater than responses to unopsonized PA ($P < 0.01$–0.001), while responses to CF PA+ derived antibodies were not significantly increased above nonopsonic baseline. Group means±SE are indicated.

increases of 224±19, 267±27, and 199±20%, respectively, compared to 131±20% by the CF PA+ antibodies ($P < 0.01–0.05$).

Discussion

The salient features of the CF opsonic defect include the following four characteristics: antigenic restriction to PA (4–6); involvement of PA LPS IgG antibodies (7, 8); a more pronounced or easily detected inhibitory effect on lung macrophages compared to blood monocytes or neutrophils (9); and inducibility after stimulation with PA LPS (14). To further characterize this phenomenon, we compared the opsonic activity of antibodies in CF PA+, CF PA−, and normal sera for nonmucoid and mucoid PA expressing LPS immunotype 1 antigens. All CF PA+ patients showed evidence of intense stimulation with PA LPS as reflected in high serum IgG antibody levels.

In addition, we considered the immune response to alginate, which is antigenically related to the exopolysaccharide slime produced in large quantities by mucoid morphotypes of PA (26–28). Recently, Pier et al. suggested that uncharacterized subsets of mucoid exopolysaccharide antibodies may be protective against PA colonization in certain CF patients. A majority of older CF patients (≥ 12 yr) without PA colonization had elevated serum opsonic titers of antibodies capable of killing mucoid PA in conjunction with PMN and complement. These antibodies seemed specific for exopolysaccharide rather than LPS. Curiously, these sera did not show elevated titers of exopolysaccharide-binding antibodies in an hemagglutination assay (30). Our studies of the opsonization of MPA provide some support to these data, in that relatively high complement-independent PMN CL responses were induced by 36% of CF PA− sera tested despite low ELISA levels of LPS and alginate IgG antibodies. However, most of the CF PA− sera in our sample population (ages 14–30 yr) showed opsonizing properties for MPA no different than those in normal control sera. It therefore seems unlikely that opsonizing antibodies for mucoid exopolysaccharide undetectable by antigen-binding immunoassays are major factors responsible for the absence of PA colonization in occasional older CF patients.

PMN play an essential role in normal systemic host defense against PA (31). Before PMN can perform their bactericidal function they must first be activated to efficiently phagocytose the microbes and undergo an oxidative respiratory burst, a process usually dependent upon the presence of antibody and/or complement opsonins. However, great heterogeneity in opsonic requirements for different strains and phenotypes of PA has been documented. Most nonmucoid or mucoid strains of PA are opsonized primarily by complement in nonimmune serum (with an additional requirement for natural antibody by some strains), whereas antibody alone is sufficient in immune serum (31–34). PA strains dependent only upon antibody for opsonization, and strains phagocytosed efficiently by PMN in the absence of any soluble opsonins, have also been described (35–37). Opsonization of our test NMPA strain was primarily antibody dependent, since PMN chemiluminescence responses were not significantly diminished after inactivation of complement. Chemiluminescence responses to this strain are similar to those Allen and Lieberman found in NMPA strains using complement-depleted rabbit immune serum opsonins (38). In contrast, the CF-derived MPA test strain was dependent upon complement for optimal opsonization by nonimmune serum, as has been reported for other MPA strains (33, 39).

Immunotype-specific IgG antibodies are directed against carbohydrate epitopes on PA LPS side chains and are potent opsonins (40). Insufficient or abnormal serum PA LPS IgG antibodies predispose to PA infections and adversely affect prognosis (41). CF patients are initially colonized with typical smooth LPS side chain-producing PA strains (42). Over time, the unique CF endobronchial environment induces or allows a number of changes in the phenotype of the colonizing PA strains, including emergence of the characteristic mucoid morphotype (due to increased exopolysaccharide production), and decreased LPS side chain production (24). Despite this phenotypic evolution, serotype-specific IgG antibodies to PA LPS persist at high titer throughout the course of the disease (19, 43, 44). Recent studies confirm persistent low-level production of LPS side chains by many CF-derived PA strains (44, 45).

Fick and co-workers reported that PA LPS IgG antibodies isolated from serum of CF PA+ patients opsonized NMPA for phagocytosis by human lung macrophages less efficiently than comparable antibodies isolated from normal immune donors. A defective interaction between the Fc antibody region and lung macrophage Fcγ receptors was implicated (7). Subsequently, they found that similar antibodies from CF bronchoalveolar lavage fluid were also defective, but in this case a localized proteolytic degradation of immunoglobulin was suggested as a major mechanism (8). However, proteolysis, while potentially contributing to opsonic dysfunction, does not adequately account for the specificity of defective CF opsonins. We therefore undertook studies to further characterize the nature of the defect.

For our opsonic studies we used blood PMN rather than lung macrophages as an easily accessible and convenient target cell to further analyze antibody-phagocyte interactions, knowing that differences between CF PA+ and CF PA− or normal serum opsonins might be more difficult to demonstrate. For example, Thomassen et al. found CF PA+ sera-induced phagocytic inhibition more pronounced with macrophages than monocytes or PMN (9). On the other hand, Holland et al. reported depressed PMN phagocytosis of rabbit antihuman immunoglobulin-coated polyacrylamide beads in the presence of CF PA+ sera (46). Since this inhibition was probably not due to immune complexes, it is possible that it represented binding of poorly opsonic CF PA+ antibodies by the rabbit antihuman immunoglobulin with subsequent depression of FcγR-mediated uptake by PMN. Second, we employed serum rather than bronchoalveolar lavage (BAL) CF opsonins, for several reasons: they are easily accessible; they are standardized in terms of other constituent proteins, in contrast to BAL where dilutional and processing artifacts are likely; and, again in contrast to BAL, they are not demonstrably fragmented. Therefore, opsonic differences between CF PA+ serum antibodies and their counterparts in CF PA− or NHS must have another basis.

Among our patients the opsonic activity of native decomplemented CF PA+ sera for nonmucoid or mucoid PA was greater than that in normal or CF PA− sera, and roughly equivalent to that of hyperimmune globulin prepared from

normal blood donors. The increased PMN chemiluminescence-inducing activity of CF PA+ sera is consistent with local containment of PA and the virtual absence of PA sepsis in CF. It also represents a potential mechanism of airways inflammation via extracellular release of reactive oxygen intermediates from local PMN (47). LeBlanc and co-workers also found increased PMN chemiluminescence induced by PA opsonized with CF PA+ sera (48). Recently, Bender et al. reported increased PMN superoxide anion production induced by CF PA+ sera (47).

These prior studies did not examine antibody specificities or content in the sera. After adjustment of chemiluminescence values for autologous PA LPS IgG antibody levels, we found that CF PA+ sera had diminished equititer opsonic activity when compared to CF PA− and normal nonimmune sera. This analysis suggested that qualitative defects in CF PA+ LPS antibodies might be present but compensated for by the pronounced, broad, and polyisotypic antibody responses that follow PA colonization.

Evidence for this hypothesis came from two sets of experiments. First, after immunoprecipitation of PA LPS antibodies from CF PA+ sera opsonic activity was enhanced in 4 of 10 sera and unaffected in two others, while that of CF PA− sera was uniformly inhibited. In contrast, there was no compelling evidence that alginate antibodies were potent opsonins; in fact, alginate-specific blocking activity appeared present in three of nine CF PA− sera tested. Second, after PA LPS IgG antibodies were isolated and purified from serum by immunoaffinity chromatography, induction of PMN CL by CF PA+ antibodies was significantly lower than that from noncolonized CF patients, normal nonimmune controls, or immune controls at equal antigen-binding antibody concentrations (Fig. 8), and at or below nonopsonic values in most cases.

In assessing the clinical significance of these findings, two crucial questions must be answered. First, since nonopsonic PA LPS-specific IgG antibodies can be induced by chronic antigenic stimulation, how do we account for the high PMN oxidative responses to viable PA opsonized by CF PA+ sera in our and other studies? Second, since CF PA+ sera do demonstrate enhanced opsonic activity, how can the presence of nonopsonic LPS antibodies contribute to PA persistence in CF airways?

In answer to the first question, it is clear from our antibody isolation and immunoadsorption experiments that in some CF PA+ sera functional opsonic LPS antibodies predominate. However, even in these sera removal of LPS antibodies only modestly depresses PMN responses when compared with the effect seen in CF PA− sera (Fig. 7). It seems reasonable to speculate that the net LPS antibody response in each individual represents the outcome of a functionally heterogeneous mixture of both opsonic and nonopsonic antibody subspecies. Such a mixture would likely vary both between individuals and over time in a given individual. In contrast to normal individuals, in most CF PA+ patients opsonic antibodies are unlikely to be LPS-specific, since PMN CL responses in these individuals do not correlate with antibody levels either in native serum or after isolation from serum. Furthermore, the adsorption and isolation data indicate that other specificities must be involved in opsonization of PA by CF PA+ sera. Therefore, candidate specificities must include other expressed surface PA antigens such as PA LPS core epitopes, exopolysaccharide, and outer membrane proteins, all of which are immunogenic in CF patients (30, 49, 50). Further studies are needed to define potential mechanisms underlying differences in opsonic function of antibodies directed against various PA antigens, such as isotype restriction determined by antigen composition and/or structure (e.g., protein versus carbohydrate).

A candidate mechanism for induction of nonopsonic antibodies is antigen-induced alteration of IgG antibody isotype (12, 13). To study the relationship between antibody isotype and opsonic function, we employed a set of isotype switch variant PA LPS specific murine monoclonal antibody clones of IgG2a, IgG2b, and IgM classes. In these experiments clear isotype-based differences in opsonic activity were demonstrated (Figs. 3 and 4). Furthermore, in preliminary studies we have found evidence that polyclonal PA LPS antibodies of different IgG subclasses isolated from human serum also show opsonic differences in vitro (manuscript in preparation) (51).

An answer to the second critical question, what role nonopsonic LPS antibodies could play in PA persistence when other opsonic antibodies compensate for their presence in PMN phagocytic studies, is not possible with the data available. In the first place, the experiments reported here do not address the potential in vivo role of pathogen-related factors such as microcolony formation or exoproteins, or the role of nonimmune host factors such as altered respiratory secretions or complement-related opsonic defects, which may be important in PA persistence. It seems more likely than not that PA persistence results from several interacting host- and pathogen-based phenomena. Secondly, our methodology also imposed inherent limitations. For example, there is likely to be a greater impact of nonopsonic LPS antibodies upon lung macrophage than PMN phagocytic activity, potentially amplifying the local effect (3, 4, 9). It is also possible that in the airways microenvironment adjacent to colonizing PA, opsonic antibodies may ineffectively compete with high local concentrations of nonopsonic LPS antibodies. If so, nonopsonic antibodies could play a role in the pathological shift in inflammatory cell predominance from macrophage to PMN seen in the CF lungs, a shift believed crucial in the evolution of progressive lung injury (52). Ineffective phagocytosis by macrophages with PA persistence could drive recruitment, activation, and degranulation of PMN with associated progression of lung disease. In later stages of the disease process, nonopsonic LPS antibodies could dampen PMN-related inflammation, contributing to the indolence and chronicity of CF lung disease.

Clearly, further investigation focusing on local in situ conditions at various stages of the disease is required to resolve current ambiguities. However, the development of nonopsonic LPS antibodies shows that the potential for a maladaptive host immune response to PA is realized in at least one antigen-specific instance, and may therefore indicate that other analogous responses also exist. In addition, this phenomenon may help explain the paradox of PA persistence and local lung inflammation existing side-by-side with decades-long chronicity, containment of infection, and intact systemic immunity to PA in cystic fibrosis.

Acknowledgments

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