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Characterization of Antibody-Mediated Inhibition of *Pseudomonas aeruginosa* Adhesion to Epithelial Cells

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An enzyme-linked immunosorbent assay system was developed and used to study adhesion of *Pseudomonas aeruginosa* to human epithelial cells and the abilities of specific antibodies to inhibit this process. Human buccal epithelial cells coated onto microtiter plates were incubated with *P. aeruginosa* suspensions, and adherent bacteria were detected by using anti-*P. aeruginosa* serum and a horseradish peroxidase-conjugated secondary antiserum. Adhesion, quantitated as an increase in A_{405}, varied linearly with increasing numbers of bacterial CFU added per well in the range of 10^5 to 10^8 CFU per well. Adhesion of *P. aeruginosa* increased following trypsinization of buccal epithelial cells. Preincubation of bacteria with monoclonal antibodies directed against *P. aeruginosa* outer membrane protein H_2 inhibited adhesion with all eight of the isolates tested. Preincubation of *P. aeruginosa* with sera from infected cystic fibrosis patients also resulted in inhibition of adhesion in the enzyme-linked immunosorbent assay system. This inhibitory activity was shown to be due to two factors: *P. aeruginosa*-specific immunoglobulin G and a non-immunoglobulin G serum component. These data support the hypothesis that bacterial components other than pili are involved in adhesion and suggest that anti-*P. aeruginosa* antibodies may be of use in preventing adhesion and subsequent colonization with *P. aeruginosa*.

Adhesion of a bacterium to the epithelial surface of a host is believed to be a necessary event that facilitates infection (2, 8, 19, 25). Thus, adhesins of *Pseudomonas aeruginosa* are considered to be important virulence determinants in respiratory infections with this organism. Johanson et al. (7) demonstrated that the ability of *P. aeruginosa* to persist in the respiratory tract is correlated with the organism's ability to adhere to the upper respiratory epithelium. Furthermore, Woods et al. (23) demonstrated that there is a correlation between in vitro adherence of *P. aeruginosa* to the upper respiratory tract epithelium and colonization of the respiratory tract with this organism.

Traditionally, two components of *P. aeruginosa* have been held responsible for adhesion of the bacterium to host epithelium, namely, pili (17, 20, 25) and alginate (5, 15). However, other components, such as protease, phospholipase C, elastase, and phenazine pigment phenotypes of the bacterium, have been shown to be associated with the adhesion process (11, 18, 24). Three recent studies have suggested that additional adhesins are also employed by *P. aeruginosa*. Doig et al. (3) were unable to inhibit *P. aeruginosa* binding to buccal epithelial cells (BECs) completely by preincubation with purified pili. In a study by Saiman et al. (18), it was shown that a *pil* mutant, strain PAK/NP, was capable of adhering to bovine tracheal epithelial monolayers in culture, although at lower levels than its parental strain. Also, in a recent study, Baker et al. (1) have demonstrated the ability of exoenzyme S on the surface of *P. aeruginosa* to function as an adhesin.

This study describes the development of an enzyme-linked immunosorbent assay (ELISA) and its use to measure the abilities of monoclonal antibodies directed against outer membrane proteins (OMPs) F and H_2 and antibodies from *P. aeruginosa*-infected cystic fibrosis (CF) patients to inhibit the adhesion of *P. aeruginosa* to human BECs.

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This approach may be useful in improving our understanding of all of the factors which are important in the establishment of colonization in *P. aeruginosa*-infected individuals.

MATERIALS AND METHODS

Blood samples. Serum samples were obtained from CF patients attending Our Lady's Hospital for Sick Children and St. Vincent's Hospital, Dublin, Ireland. Control serum samples were obtained from healthy laboratory volunteers and from the National Blood Transfusion Service Board. All samples were aliquoted and stored at −20°C. Each CF patient had evidence of *P. aeruginosa* colonization (at least two consecutive positive sputum cultures for *P. aeruginosa* before blood samples were taken) and a high anti-*P. aeruginosa* antibody titer in serum as determined by immunoblotting (21).

Bacteria. *P. aeruginosa* cultures used in this study were sputum isolates from CF patients attending Our Lady's Hospital for Sick Children. Bacteria were stored on tryptone soya agar slopes at 4°C. Bacterial isolates were cultured overnight on tryptone soya agar. Control bacterial isolates used included clinical isolates of *Escherichia coli*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. Bacteria were harvested into phosphate-buffered saline (PBS) (10 mM phosphate, 150 mM NaCl) containing 1 mM $MgCl_2$ (PBS-Mg) (25) by using sterile cotton swabs and diluted as required in PBS-Mg. Bacterial cells were fixed by incubation in 0.3% formaldehyde overnight and washed three times by using sterile PBS.

BECs. BECs from healthy adult volunteers were collected into PBS after vigorous rubbing of buccal mucosa with sterile cotton swabs. Cells from all volunteers were pooled, washed six times (by centrifugation at 1,000 × *g* and suspension in PBS), and finally suspended to the required concentration in PBS-Mg (25).

Preparation of rabbit antisera. New Zealand White adult rabbits were immunized with *P. aeruginosa* outer mem-

brane, cytoplasmic and extracellular extracts (21), or formalinized whole cells by using the immunization protocol of Owen (14). For the experiments described, pooled serum from rabbits immunized with each of the immunogens was used.

Monoclonal antibodies. Monoclonal antibodies with specificity for *P. aeruginosa* OMPs F [H_4(MA4-4)] and H_2 [F_6(MAI-6)] were crude ascites preparations at protein concentrations of 1.5 and 2.5 mg/ml, respectively (kindly provided by G. Crockford and R. E. W. Hancock, Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada).

Coating of *P. aeruginosa* onto microtiter wells. *P. aeruginosa* suspensions at dilutions of 10^3 to 10^9 CFU/ml were coated onto microtiter wells by using 0.1 M carbonate-bicarbonate buffer, pH 9.6. Volumes of 100 μl were added to microtiter wells in triplicate. After incubation for 3 h at 37°C, the wells were washed three times with PBS. Bacteria bound to wells were fixed by using formaldehyde (0.3% in PBS) and immunodetected as described below for the adhesion assay.

Adhesion assay. The adhesion assay system used was based on the assay described by Ofek et al. (13). Microtiter plates (Dynatech M29B) were coated with 100 μl of poly-L-lysine (50 μg/ml) at 37°C for 30 min and then washed twice with PBS. BEC suspensions (100 μl at 10^5 cells per ml) were then added to the plates. After 10 min at room temperature, the plates were centrifuged at 1,000 × *g* for 10 min. The supernatants were carefully aspirated by using a multichannel pipette, and the plates containing a monolayer of BECs were dried (by overnight incubation at 37°C) and stored for up to 2 weeks at room temperature. BECs bound to microtiter wells were fixed by incubation with 0.25% glutaraldehyde for 10 min. After this and in all of the subsequent steps in the assay, the wells were washed four times with PBS. Nonspecific binding of bacteria to microtiter wells was blocked by incubation with gelatin (2 mg/ml in PBS) for 1 h at room temperature. *P. aeruginosa* or control bacterial suspensions (100 μl at 10^9 CFU/ml in PBS-Mg) were added to the BEC-coated wells, and the plates were incubated at 37°C for 1 h. The adherent bacteria were fixed by overnight incubation with 0.3% formaldehyde in PBS. Gelatin (2 mg/ml in PBS) was added as a blocking agent during immunodetection. Pooled rabbit anti-*P. aeruginosa* serum was added to wells at a 1/500 dilution and followed 1 h later by biotinylated goat anti-rabbit immunoglobulin G (IgG) and a streptavidin-biotinylated horseradish peroxidase complex (Amersham). Color development of *ortho*-phenylenediamine substrate was measured as A_{405} after 30 min by using a microplate autoreader. All samples were assayed in triplicate.

Inhibition of adhesion. The ability of *P. aeruginosa*-specific monoclonal antibodies, CF serum, or serum fractions to inhibit adhesion in this model system was assayed as follows: potential inhibitors of adhesion were preincubated at 37°C for 1 h with *P. aeruginosa* suspensions diluted in PBS-Mg to a final bacterial concentration of 10^9 CFU/ml; bacteria were then added directly to BEC-coated wells, and adhesion was assayed as before.

Treatment of BECs with proteolytic enzymes. BEC-coated microtiter plates (glutaraldehyde fixed) were incubated with trypsin (from porcine pancreas, type IX [EC 3.4.21.4., 1,740 BAEE U/g of solid]), α-chymotrypsin (from bovine pancreas, type II [EC 3.4.21.1., 51 U/mg of protein]), protease (from *Streptomyces griseus*, 150 U/g of solid), or pepsin (from porcine stomach mucosa [EC 3.4.23.1., 107 U/mg of solid]) solutions in PBS at fivefold dilutions from 1,000 to 0.128 μg/ml of protein for 10 min at 37°C. The reactions were stopped by washing the plates six times with PBS. Adhesion assays were then carried out on treated wells as described above.

Fractionation of human serum by protein A affinity chromatography. Purification of IgG from human serum was done by using a protein A-Sepharose 4B column, run at 4°C, at a flow rate of 5 ml/h. Serum samples diluted 1/2 in 0.1 M phosphate buffer, pH 8.0, were applied to a 1-ml column. After the column was washed with 10 ml of phosphate buffer, the bound IgG was eluted by using 0.1 M sodium citrate, pH 3.5, and collected as 1-ml fractions into tubes containing 60 μl of 2 M Tris. Collected fractions were dialyzed overnight against PBS and assayed for protein by measuring A_{280}.

Absorption of *P. aeruginosa* adhesion-inhibitory factors. *P. aeruginosa* suspensions containing 10^{10} CFU in PBS-Mg were pelleted by centrifugation in Eppendorf tubes. The supernatants were removed, and the pelleted bacteria were suspended to 1 ml with CF serum, CF IgG, or IgG-depleted serum (obtained following protein A affinity chromatography). After incubation at 37°C for 1 h, the bacteria were pelleted by centrifugation in a Biofuge. The supernatants obtained were assayed by the adhesion ELISA system for the presence of adhesion-inhibitory factors.

RESULTS

Optimization of assay conditions. To determine optimal conditions for immunodetection of bacteria in the adhesion assay system, *P. aeruginosa* (isolate P103) was coated directly onto microtiter wells at 10-fold dilutions by using either fresh or formaldehyde-fixed bacteria. Rabbit anti-*P. aeruginosa* serum or serum pooled from eight *P. aeruginosa*-infected CF patients with specific anti-*P. aeruginosa* antibody was used for immunodetection of bacteria. The A_{405} increased linearly over a range of 10^6 to 10^8 CFU coated per ml (Fig. 1a). Comparable A_{405} values resulted when fresh or formaldehyde-treated bacteria were used, although lower background signals were obtained with fixed bacteria. Serum from CF patients and rabbit anti-*P. aeruginosa* serum were found to be equally sensitive for immunodetection of bacteria (Fig. 1b). Rabbit anti-*P. aeruginosa* serum was unreactive with *E. coli* and *K. pneumoniae* but showed low levels of reactivity with *S. pyogenes* coated onto microtiter wells (data not shown).

Adhesion of *P. aeruginosa* to BEC-coated wells varied linearly with increasing bacterial numbers added per well in the range of 10^6 to 10^9 CFU per well. Figure 2 shows the results for seven isolates tested. The signal intensity for individual isolates was quite variable. Owing to the nature of the assay system, this observed variability may have been due to (i) variability in the ability of the *P. aeruginosa* strains to react with the anti-*P. aeruginosa* serum used for immunodetection, (ii) variability in the ability of the *P. aeruginosa* strains to adhere to BECs in BEC-coated microtiter plates, or (iii) some combination of the two. To determine which of these effects contributed to the signals obtained in the adhesion ELISA, the following experiments were carried out. Suspensions of 20 *P. aeruginosa* isolates, containing 10^9 CFU/ml, were coated directly onto microtiter wells by using a carbonate-bicarbonate buffer system, and bound bacteria were immunodetected by using anti-*P. aeruginosa* serum (see Materials and Methods). Suspensions of the same isolates in PBS were incubated with BECs coated onto microtiter wells, and bacteria adhering to BECs were detected by using the same anti-*P. aeruginosa* serum. Table 1

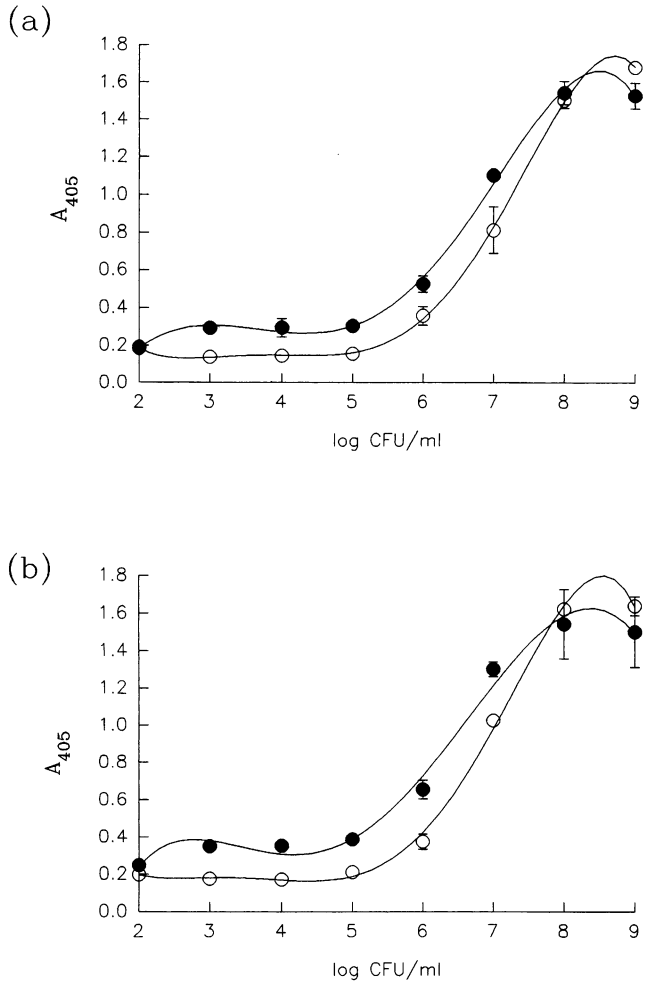

FIG. 1. Immunodetection of *P. aeruginosa* in an ELISA system. *P. aeruginosa* (isolate P103) was coated onto microtiter wells at increasing concentrations (10^2 to 10^9 CFU/ml). Bound bacteria were incubated with either pooled rabbit anti-*P. aeruginosa* serum (1/500 dilution) (a) or pooled plasma from *P. aeruginosa*-infected CF patients (1/1,000 dilution) (b). Biotinylated secondary antisera and a streptavidin-biotinylated horseradish peroxidase complex with *ortho*-phenylenediamine as the substrate were used for immunodetection. Numbers of bacterial CFU are plotted against A_{405}. Each point represents the mean ± standard deviation of triplicate wells of a representative experiment carried out three times. The experiment was done with fresh (●) and formaldehyde-fixed (○) bacteria.

shows the results of this experiment. Certain isolates were immunoreactive with the rabbit antiserum used, while others were not. Absorbance values in wells in which comparable numbers of bacteria were coated varied from 0.18 (isolate P27) to 1.46 (isolate P6) absorbance units, demonstrating variability in the ability of the anti-*P. aeruginosa* serum used to react with individual *P. aeruginosa* isolates. Immunodetection of bacteria adhering to BECs resulted in A_{405} values of 0.11 (isolate P10) to 1.18 (isolate P26) in the adhesion assay. Thus, individual isolates varied greatly, in both immunoreactivity with the rabbit anti-*P. aeruginosa* serum used and the ability to adhere to BECs. For example, three isolates with comparable, low A_{405} values of 0.17, 0.16, and 0.18 U (P11, P20, and P23, respectively) in the adhesion assay, showed a large variation in absorbance values when

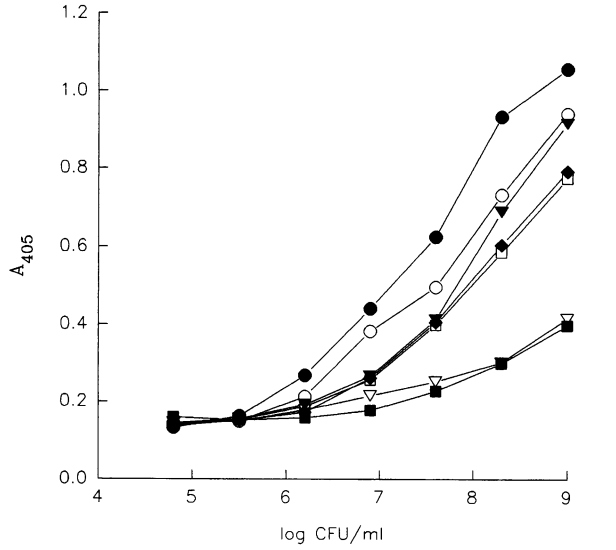

FIG. 2. Immunodetection of individual *P. aeruginosa* isolates adhering to BECs. Plot showing the effect of increasing *P. aeruginosa* numbers (fivefold dilutions from 6.4×10^4 to 1×10^9 CFU/ml) added to BEC-coated wells (coated at 10^4 cells per well) on adhesion, measured as increasing A_{405}. Results are shown for seven *P. aeruginosa* isolates, i.e., P6 (●), P7 (○), P10 (■), P12 (□), P13 (▼), P14 (▽), and P36 (◆).

bacteria coated directly onto wells were immunodetected (1.22, 0.70, and 0.47 absorbance units, respectively).

Protease treatment of BECs. Following treatment of BEC-coated microtiter wells with proteolytic enzymes, no change

TABLE 1. Comparison of the ability of rabbit anti-*P. aeruginosa* serum to detect isolates with results of adhesion assays

Isolate	A_{405}	
	P. aeruginosa coated to plate[a]	BECs incubated with *P. aeruginosa*[b]
P5	0.97	0.15
P6	1.46	0.74
P7	1.40	0.90
P8	1.07	0.66
P9	1.22	0.58
P10	0.87	0.11
P11	1.22	0.17
P12	1.02	0.83
P13	0.97	0.72
P14	1.12	0.58
P15	1.16	0.62
P20	0.70	0.16
P23	0.47	0.18
P26	1.02	1.18
P27	0.18	0.24
P35	1.43	0.14
P36	1.21	0.46
P84	1.34	0.51
P85	1.34	0.55
P103	1.40	0.17

[a] Bacterial suspensions coated directly onto microtiter plates by using a carbonate-bicarbonate buffer system were immunodetected with rabbit anti-*P. aeruginosa* serum.

[b] Bacterial suspensions were added to BEC-coated microtiter wells, and adherent bacteria were immunodetected by using rabbit anti-*P. aeruginosa* serum.

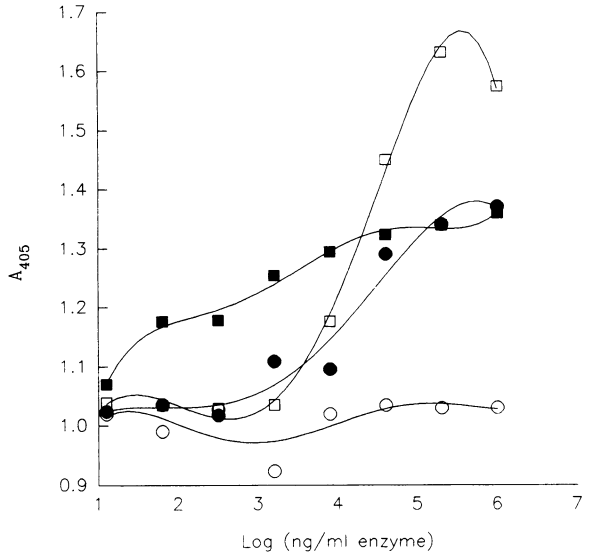

FIG. 3. Proteolytic enzyme treatment of BECs—effect on adhesion of *P. aeruginosa*. Plot showing the effects of increasing concentrations of the proteolytic enzymes trypsin (□), α-chymotrypsin (■), protease (●), and pepsin (○) (0.0128 to 1,000 μg/ml), used to pretreat BECs coated onto microtiter wells, on adhesion measured as A_{405}. *P. aeruginosa* was used at a concentration of 10^9 CFU/ml.

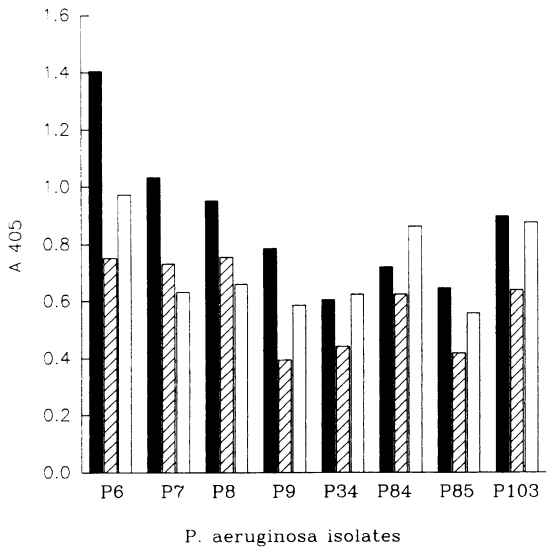

FIG. 4. Effects of *P. aeruginosa*-specific monoclonal antibodies on adhesion of *P. aeruginosa* to BECs in an ELISA system. *P. aeruginosa*-specific monoclonal antibodies H_4 (□) and F_6 (▨) (or PBS as a control [■]) were preincubated with *P. aeruginosa* (final concentration, 10^9 CFU/ml) and then assayed in the adhesion ELISA system. Results are shown for the eight *P. aeruginosa* isolates tested (P6, P7, P8, P9, P34, P84, P85, and P103).

in the numbers of BECs bound per well was evident by microscopy. Pretreatment of BECs with trypsin (1.6 to 200 μg/ml) resulted in a linear increase in adherence of *P. aeruginosa* to BECs (Fig. 3). In contrast, pepsin treatment of BECs had no effect over the range of concentrations used. Protease and α-chymotrypsin treatments of BECs also caused increased adherence over the range of concentrations examined, although the effects were not as pronounced as those observed with trypsin.

Inhibition of adhesion by monoclonal antibodies. Two *P. aeruginosa*-specific monoclonal antibodies were examined for the ability to inhibit adhesion of *P. aeruginosa* isolates to BECs in the ELISA system. Eight *P. aeruginosa* isolates were used in this experiment. Each of the monoclonal antibodies, at a 1/10 dilution of original stock preparations, was preincubated with *P. aeruginosa* prior to use in the adhesion assay. Net absorbance values for isolates pretreated with monoclonal antibodies were compared with those obtained when isolates were incubated with PBS (Fig. 4). The most effective inhibitor of adhesion with all eight *P. aeruginosa* isolates tested was F_6(MA1-6), which resulted in 13 to 50% decreases in absorbance readings with the eight *P. aeruginosa* isolates tested. Monoclonal antibody H_4(MA4-4) partially inhibited adhesion of five of eight isolates to the BECs.

Inhibition of adhesion using serum from CF patients and healthy individuals. Serum samples from 10 healthy individuals and 12 CF patients with *P. aeruginosa* infection were examined for the ability to inhibit adhesion in the ELISA system. Fivefold dilutions of sera were used, starting with a 1/10 dilution. Results of this experiment (Fig. 5) demonstrate the relative potency of the CF sera compared with control sera as inhibitors of adhesion. Differences in adhesion (A_{405}) between bacteria treated with CF and control sera were most pronounced at high serum dilutions. With both control and CF sera, adhesion increased with increasing serum dilutions over the range of 1/10 to 1/31,250.

Investigation of the nature of serum inhibitory activity. CF serum, pooled from 10 of the above-described donors, was fractionated on a protein A-Sepharose 4B column to isolate IgG antibodies. The column fractions were assayed to deter-

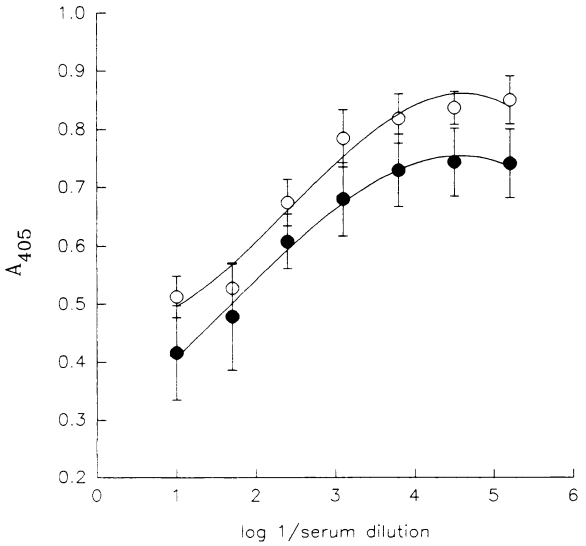

FIG. 5. Inhibition of adhesion by sera from *P. aeruginosa*-infected CF patients. Plot showing adhesion of *P. aeruginosa* to BECs following preincubation of bacteria with sera from 12 individual CF patients with *P. aeruginosa* infections (●) and 10 healthy controls (○) (mean [± 1 standard deviation] A_{405} units for each study group). A fivefold dilution of each serum was used.

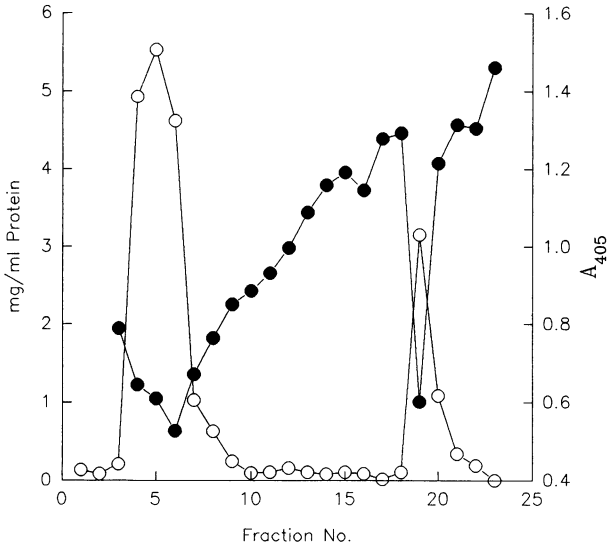

FIG. 6. Adhesion-inhibitory activity of CF serum components separated on a protein A affinity column. Protein elution profile (ng/ml) (○) and *P. aeruginosa* adhesion-inhibitory activity (A_{405}) (●) of each of the 1-ml fractions collected from a protein A affinity column.

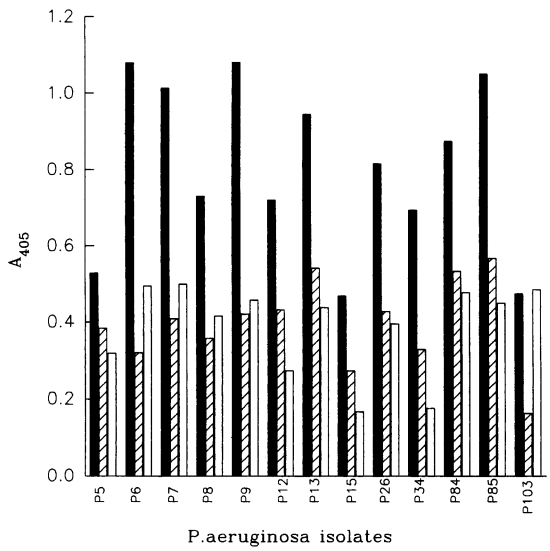

FIG. 7. Effects of serum adhesion-inhibitory factors on *P. aeruginosa* isolates. The IgG fraction (▨) and IgG-depleted serum fraction (□) from a protein A column separation of pooled CF serum were adjusted to the same protein concentration (10 mg/ml) and assayed alongside *P. aeruginosa* isolates (incubated in the absence of an inhibitor) (■) for the ability to inhibit adhesion of 13 *P. aeruginosa* isolates (P5, P6, P7, P8, P9, P12, P13, P15, P26, P34, P84, P85, and P103) at 10^9 CFU/ml to BECs in an ELISA.

mine their effects on adhesion by using *P. aeruginosa* isolate P7. Decreasing A_{405} values represent inhibition of adhesion. Figure 6 shows the protein concentrations of fractions and the corresponding A_{405} units from adhesion assays. The two major protein peaks present, at fractions 6 and 19, corresponded to the two peaks of adhesion-inhibitory activity in the fractions. The distribution of immunoglobulins in the fractions was established by staining immunoblots of fractions with antisera to human IgG, IgA, and IgM (21). Most of the IgG was present in fractions 19, 20, and 21. Trace amounts of IgG were also detected in the main serum protein peak (fractions 4 to 8). IgA was present in all fractions from 3 to 21, although it was most concentrated in the serum protein peak. IgM was detected in serum protein fractions 4 to 8 and 19 (data not shown). Fractions from the two protein peaks which inhibited adhesion were pooled and adjusted to equal protein concentrations (10 mg/ml). The effects of these preparations on the adhesion of 13 *P. aeruginosa* isolates to BECs were then examined. Both the IgG fraction and the IgG-depleted serum fractions were effective inhibitors of adhesion with each of the 13 *P. aeruginosa* isolates examined (Fig. 7). The extent of inhibition for individual isolates was variable. In some cases (e.g., isolate 34), the IgG-depleted serum fraction was a more potent inhibitor of adhesion than the IgG fraction; in some cases, the reverse was true (e.g., isolate 6).

Pooled fractions used in the above-described experiment, at a uniform protein concentration of 10 mg/ml, were absorbed with freshly cultured *P. aeruginosa*, and the absorbed fractions were assessed for adhesion-inhibitory activity as before. The percentages of inhibition achieved by using CF serum, IgG-depleted serum, and IgG fractions were compared pre- and postabsorption with that achieved with a PBS buffer control (Table 2). Absorption of unfractionated CF serum by *P. aeruginosa* decreased its ability to inhibit adhesion by half. In the case of the IgG fraction, at 10 mg of protein per ml, all adhesion-inhibitory activity was lost following absorption. The IgG-depleted serum fraction, also at 10 mg of protein per ml, was unchanged by absorption with *P. aeruginosa*.

DISCUSSION

Identification of adhesins used by pathogenic bacteria to bind to host epithelium and immunization of susceptible hosts with these adhesins have been used successfully as an approach to disease prevention in the veterinary field. Adhesin vaccines are now used for protection of animals against enterotoxigenic *E. coli* and have been used in trials with humans (9). Various systems have been used to mimic the adhesion of *P. aeruginosa* to epithelial cell surfaces. Adhesion models described include mouse tracheal rings (17) and BECs (25), used to study colonization of the airways; the SIRC cell line used to study *P. aeruginosa* corneal infection (6); and mouse epidermal cells, used to study adhesion to burned skin surfaces (20). In studies on

TABLE 2. Effect of absorption of CF serum and serum fractions on adhesion-inhibitory activity

Adhesion inhibitor[a]	Absorption with *P. aeruginosa*	% Inhibition of adhesion
None (PBS control)	−	0
CF serum	−	54
CF IgG	−	60
CF IgG-depleted serum	−	51
CF serum	+	27
CF IgG	+	0
CF IgG-depleted serum	+	62

[a] CF serum was used at a dilution of 1/10, and serum fractions were adjusted to 10 mg of protein per ml.

respiratory infection, both normal cells (12) and cells damaged by acid injury (16) or trypsinization (23) have been examined to identify factors important in the adhesion process in CF patients.

The ELISA system described provides a sensitive means of measuring adhesion of *P. aeruginosa* to BECs in a quantitative assay which can accommodate numerous samples per assay and allow the study of factors that inhibit or enhance the adhesion process. The increase in adhesion of *P. aeruginosa* cells to BECs following trypsinization parallels the results of Woods et al. (23), who demonstrated that increased numbers of ^{14}C-labelled *P. aeruginosa* cells adhere to BECs when cell surface fibronectin is removed by trypsinization. This suggests that the conditions under which the bacteria bind to BECs are comparable for both assay systems. Other proteases examined were not as effective as trypsin, demonstrating the specificity of this process.

The adhesion ELISA had a limiting feature in that significant variability in signal intensity for individual isolates was observed. Variability in the assay system may be due to a number of factors. It was demonstrated that the anti-*P. aeruginosa* serum used for immunodetection of bacteria did not react equally well with all of the *P. aeruginosa* isolates used, as determined by direct coating of *P. aeruginosa* to microtiter wells and immunodetection. It is possible that unequal binding of the 20 *P. aeruginosa* strains to the plastic wells contributed to the results obtained; however, it seems more likely that variable immunoreactivity occurs. For strains which react strongly with the antiserum used, the assay system is useful. Alternative high-titer anti-*P. aeruginosa* sera could be used to study adhesion of strains nonreactive in the system described. Thus, with the assay system described, strain-to-strain comparisons cannot be made unless one corrects for reactivity of the antiserum with each bacterial strain. However, this assay system could be used to examine the properties of individual strains and to examine the effectiveness of potential inhibitors of adhesion.

When the ELISA conditions were optimized, the system was shown to be quantitative. The A_{405} increased linearly with the concentration of *P. aeruginosa* (number of CFU added per well) in the range of 10^6 to 10^9 CFU/ml. The assay detection system was shown to be specific, since other bacteria coated to ELISA plates, such as *S. pyogenes* and *E. coli*, did not give rise to any signal when the anti-*P. aeruginosa* serum was used. *K. pneumoniae* showed some reactivity with antisera used for detection of *P. aeruginosa*, but this was low compared with that obtained with *P. aeruginosa*. Thus, normal flora present on the BECs used in this assay should not affect the immunodetection system used in the assay.

In all of the experiments described in which a specific antibody was used to inhibit adhesion, whole antibodies (present in whole serum, fractionated serum, or monoclonal antibody preparations) were used rather than Fab fragments (3). No agglutination of *P. aeruginosa* was evident, although small agglutinins may have been present. Use of whole antibody more closely represents the in vivo situation, in which a plethora of immune defense mechanisms may be called upon to assist in elimination of bacteria. The feasibility of inhibiting *P. aeruginosa* adhesion to BECs by using *P. aeruginosa*-specific antibodies was demonstrated in this study. The variability in the extent of inhibition attained by using eight *P. aeruginosa* isolates demonstrates the need to screen large numbers of *P. aeruginosa* isolates to establish the usefulness of particular antibodies for prevention of adhesion. The F_6 monoclonal antibody, which partially inhibited adhesion (13 to 50%) of all of the *P. aeruginosa* isolates tested, is specific for *P. aeruginosa* OMP H_2. This OMP has been shown to be surface exposed (10). Additionally, the level of H_2 expression in *P. aeruginosa* has been shown to be variable (4), which would explain the ability of this monoclonal antibody to inhibit *P. aeruginosa* to various degrees with the isolates tested. OMP F, with which monoclonal antibody H_4 reacts, caused some inhibition of adhesion of five of eight *P. aeruginosa* isolates. OMP F has been shown to be expressed in equal amounts in 30 *P. aeruginosa* clinical isolates (4); thus, it is not clear why this antibody caused decreases in the observed adhesion for some of the isolates used and not others. However, these data support the hypothesis that adhesins other than pili and alginate (strains used in this study were nonmucoid) are involved in adhesion of *P. aeruginosa* to epithelial surfaces. Recently, OMPs from Vero cytotoxin-producing *E. coli* O157:H7 strain CL-56 have been shown to inhibit adherence to epithelial cells in a competitive manner (22), further supporting the concept that nonpilus bacterial components, such as OMPs, may function in *P. aeruginosa* as adhesins.

Having demonstrated the ability of *P. aeruginosa*-specific monoclonal antibodies to inhibit adhesion in this system, we screened CF sera, known to contain *P. aeruginosa*-specific antibodies, for adhesion-inhibitory activity. Sera from both CF patients and controls were shown to contain factors which inhibited adhesion of *P. aeruginosa* to BECs. Following protein A affinity purification of pooled CF serum, it was clear that two serum adhesion-inhibitory factors are present in CF serum. One of these factors was present in the IgG fraction from the protein A column, and the second was in the main serum protein fraction, which did not have affinity for protein A. Following absorption of the CF serum with *P. aeruginosa*, inhibitory activity was reduced from 54 to 27% (Table 2). When the IgG fraction was absorbed with *P. aeruginosa*, total loss of inhibitory activity suggested that the inhibitor in this material was *P. aeruginosa*-specific IgG antibody. The nature of the second inhibitory factor in the protein A unbound fraction, which could not be absorbed by *P. aeruginosa* under the conditions described, is not obvious. Failure to remove this factor from IgG-depleted serum by absorption suggests that it is a nonspecific inhibitory effect of a serum protein, which probably interacts with the BECs rather than the *P. aeruginosa* in the adhesion assay.

Results of this study suggest that it may be possible to inhibit adhesion by using *P. aeruginosa*-specific antibodies. It seems that the presence of such antibodies in patients with CF affords no protection against colonization with *P. aeruginosa*. There are two possible reasons why this may be. The serum antibodies with adhesion-inhibitory activity were of the IgG class and therefore may not be effective at the epithelial mucosa. Second, potent IgG antibody responses to *P. aeruginosa* in CF patients are observed only in patients with established infections. Thus, the production of antibodies may be too late to afford protection to CF patients against infection. It would be useful to determine whether secretory IgA at the mucosal surfaces of individuals infected with *P. aeruginosa* has adhesion-inhibitory activity.

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