Antibody to Multiple Mucoid Strains of *Pseudomonas aeruginosa* in Patients with Cystic Fibrosis, Measured by an Enzyme-Linked Immunosorbent Assay

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ABSTRACT. The sera from 32 patients with cystic fibrosis who were chronically colonized with mucoid strains of *Pseudomonas aeruginosa* (MPA) were tested for anti-MPA antibodies. Using an enzyme-linked immunosorbent assay we measured IgA, IgG, and IgM antibody titers to three MPA strains, extracts of those strains, and seaweed-derived sodium alginate, which is similar chemically to the exopolysaccharide of MPA. These titers were compared with identical tests performed on the sera of eight cystic fibrosis patients who never were colonized with MPA and 10 normal adults. The IgG titers were significantly higher in tests of sera from the colonized patients compared with the other two groups but the IgA and IgM titers were not significantly higher. In colonized patients antibody titers to the different antigens correlated with each other suggesting that the major antibody response was to common antigenic determinants. Using these titers as a data base, eight patients whose clinical status was unknown to the testers, had IgG-enzyme-linked immunosorbent assay tests of their sera and the four colonized patients with cystic fibrosis were correctly identified. Three of them had substantial titers of antibody in the bronchoalveolar lavage fluid. (*Pediatr Res* 20:1085-1090, 1986)

bronchiectasis. PA is one of the most frequently encountered bacterial pathogens in the respiratory tracts of patients with CF. Chronically infected patients generally have PA strains recovered which have a characteristic mucoid morphology when cultivated on agar media (1). For reasons that are still obscure, carriage of MPA generally cannot be eradicated with antibiotics and once established, colonization is lifelong. Thus prevalence of MPA carriage rises with age. A worse prognosis for CF patients and an increased severity of the characteristic pulmonary disease have been associated with infection with MPA (1-3).

Previous studies have demonstrated high-titered serum antibody to PA in patients with CF and it is clear that carriage persists despite a high prevalence of antibody to whole organisms (3-5), exotoxin (6), PA proteins (7), and to the sodium alginate exopolysaccharide isolated from MPA (8-10). Antibody to specific extracts or purified antigens has generally been measured to material extracted from a single strain and little is known about whether antibody from patients infected with MPA binds equally well with extracts from other MPA isolates. In this investigation an ELISA has been used to measure isotype-specific binding antibody to 1) three MPA strains, having different Fisher immunotype antigens, 2) extracts of these strains, and 3) sodium alginate.

Abbreviations

CF, cystic fibrosis
PA, *Pseudomonas aeruginosa*
MPA, mucoid strains of PA
ELISA, enzyme-linked immunosorbent assay
BAL, bronchoalveolar lavage fluid
KDO, 2-keto-3-deoxyoctonate
PBS, phosphate-buffered saline
LPS, lipopolysaccharide

METHODS

Strains of PA. MPA strains were isolated from the sputum cultures of patients with CF seen at the Cystic Fibrosis Clinic at Yale University School of Medicine, New Haven, CT and were identified as such in the Clinical Microbiology Laboratory at Yale-New Haven Hospital. Single colonies of the strains were passed onto deoxycholate agar and stored frozen in skim milk at -70° C as well as lyophilized in skim milk. The nonmucoid variants of these strains were typed by agglutination with rabbit antisera to the seven Fisher immunotypes (11, 12). Strains stored by lyophilization have been demonstrated by a photographic record in our laboratory to retain mucoid morphology and immunotype characteristics for many years. We chose for this study three stable strains of three different immunotypes 113M (a type 1), 241M (a type 6), and 256M (a type 2). Freshly reconstituted, previously lyophilized organisms were used for each phase of the study.

Patient specimens. Patient sera for the analysis of subjects with CF from Yale University School of Medicine were casual blood specimens taken from 1978 to 1985 using a protocol approved by the Human Investigation Committee. They were taken at the time of routine clinic visits and during hospitalizations for exacerbations of pulmonary disease. The sera were stored at -70°

CF is an autosomal recessive multisystem disease in which the pulmonary component is characterized by progressive pulmonary deterioration due to chronic bronchiolitis and disabling

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C until thawed for this study. Patient data were extracted from clinic charts. Throat swab or sputum culture is a routine part of all clinical visits and all potential pathogens are speciated. Length of MPA carriage was determined by review of culture reports or reported as "longer than..." for patients who were colonized with MPA at their first clinic visit. These patients were not included in the data analysis for correlation of length of colonization with titer of antibody.

For the blinded study of University of Iowa patients numbered specimens which had been preserved at -70°C were sent on ice. They were from patients with CF followed at least 3 yr at the University of Iowa Cystic Fibrosis Center, as well as normal subjects and adult pulmonary patients having bronchoalveolar lavage by the Pulmonary Disease Division at that institution. Bronchial washings were performed on eight patients with various diagnoses. They were clinically stable at the time of the lavage and specimens were processed as published by Merrill *et al.* (13). Total protein was determined by Coomassie brilliant blue absorbance (14) and was 0.8 to 1.6 mg/ml after concentration 10- to 20-fold using positive pressure ultrafiltration (Diaflo YM5 membrane, Amicon Corp., Scientific Systems Division., Lexington, MA).

Soluble antigens. Extracts of strains 113M, 256M, and 241M were made from 200 streaked deoxycholate agar plates incubated 24 h at 37°C . The bacterial mass was scraped off each plate into 200 ml of 0.8 M EDTA. This material was cooled and homogenized in an omnimixer for 60 s and centrifuged at $30,000 \times g$ for 3 h. The clear supernatant (~ 200 ml) was dialyzed extensively at 4°C with three 10-liter bath changes against deionized water for 3 days. Carbohydrate determinations by the phenyl sulfuric acid method showed 0.2–0.5 mg/ml carbohydrate and the 256M extract which was representative of the three extracts was by weight 11% carbohydrate, 39% protein, and had trace lipopolysaccharide (determined by measurement of KDO) and nucleic acid (determined by UV spectral analysis at 280 and 260 nm).

Sodium alginate which was used for sensitizing microtiter plates for testing for antibody to alginate was obtained as a purified powder from the Fisher Scientific Company and was used as a 1% solution in deionized water.

ELISA. This assay was performed by a modification of an established technique (15). The test was run in 96-well polyvinyl chloride microtiter "U" well plates (Dynatech Laboratories, Alexandria, VA). For tests of antibody to whole bacteria the strain was grown overnight on deoxycholate agar and the bacteria were suspended in PBS at an optical density of 0.600 at 750 nm. Fifty μl of bacterial suspension were dispensed in each well and the plate was centrifuged at 1500 rpm for 5 min and then fixed with 0.25% glutaraldehyde.

For sensitization of microtiter plates with soluble extracts or with sodium alginate the optimally sensitive concentration of antigen was determined by checkerboard dilution of antigen against 2-fold dilutions of a standard rabbit antiserum to the test strain. Twenty-five μl of diluted antigen was dispensed into each well, incubated 1 h at 37°C , blocked twice with a blocker containing 5 g of casein, 5 g bovine serum albumin, 0.1 g phenol red, and 1.8 g sodium azide per liter, washed with PBS and stored at 4°C until used for an ELISA.

When an ELISA was performed, the specimen to be tested (primary antibody) was diluted in blocker and 25 μl of the appropriate dilution was dispensed into the well. A 2-fold dilution scheme from 1:10 to 1:327,680 was used. The plate was then incubated at room temperature at least 4 h, and the serum was aspirated from the wells. The plate was blocked, washed, with PBS and then 25 μl of α , μ , or γ chain-specific, alkaline phosphatase-conjugated goat antihuman immunoglobulin (Tago, Inc., Burlingame, CA) diluted to 1:2000 was added to the well and incubated at least 2 h at room temperature. The enzyme-conjugated serum was aspirated from the wells and the plate was again washed with PBS. The test was developed by adding 50 μl of an alkaline (pH 9.8) solution of *p*-nitro phenyl phosphate (1

mg/ml) in a diethanolamine buffer at room temperature for 30 min and stopping the reaction with 25 μl of 3 N NaOH. The intensity of the color reaction was read using a Titer-Tec spectrophotometer (Flow Laboratories, McLean, VA) at 405 nm absorbance. The titer of each serum was defined as the reciprocal of the highest dilution resulting in an optical density of ≥ 0.100 using as a negative control and internal zero standard a well in which the primary antibody is replaced by blocker.

In all tests reported, this control was colorless to the naked eye, while wells with $\text{OD} \geq 0.070$ were obviously yellow compared with a control containing no substrate. In a separate experiment, a control without substrate was added and when the spectrophotometer was zeroed on this well; the controls without the primary antibody read < 0.2 and the test sera showed no difference in titer or OD. The positive control was an antibody-containing normal human serum. The titer of this serum was always within a 1- to 2-fold dilution of a previously run control experiment. When the reproducibility of titrating this serum (Table 3, subject 6) was analyzed on the basis of the number of 2-fold dilutions of a standard 1:10 dilution, results were as follows for seven tests on different days. For 256 whole bacteria, 6.14 ± 0.69 (titer 1:160–1:320), coefficient of variation 11%; 256 extract 5.0 ± 0.00 (titers 1:160, coefficient of variation 0%), 113 extract 5.29 ± 0.76 (titers 1:80–1:320, coefficient of variation 14%).

In the absorption experiment the ELISA was performed exactly as described except the serum was preabsorbed with whole MPA (12). The absorbed and unabsorbed portions of the serum specimen were handled identically and were tested at the same time on the same microtiter plates.

Statistical analysis. Correlation between serum titers against each pair of ELISA antigens and between titers and length of colonization was tested using the Pearson coefficient of correlation (*r*) using a programable calculator (Hewlett Packard HP 25). The level of significance reported was determined using Colton's (16) tables. Geometric means of the three subject groups were compared using the two-tailed *t* statistic for two means (17). Differences between groups were considered to be statistically significant if $p \leq 0.05$.

RESULTS

Antibody to MPA in the sera of CF patients and normal subjects. Tables 1–3 show the serum IgG-ELISA titers for 32 patients with CF who were colonized with MPA, eight CF patients never colonized with MPA, and 10 normal young adults.

It can be seen in Table 1–3 that geometric mean titers for the colonized patients with CF were much higher than for the other two subject groups. The significance of this difference was determined using the two-tailed *t* statistic for two means and is shown in Table 4. The colonized patients with CF had significantly more antibody to the three strains of MPA, the extracts from the three strains and to sodium alginate, compared with either the uncolonized patients or the normals. The sera of the latter two subject groups had titers that were not significantly different from each other. When IgM-ELISA titers and IgA ELISA titers were compared for these two groups, mean titers did not differ significantly using $p \leq 0.05$ as a significance level. As shown in Table 5, the mean titers of IgA antibody were substantially higher for the colonized CF group than for the other two groups but the spread of the titers and the size of the groups were responsible for *p* values greater than 0.05. The IgM titers were comparatively low in all three groups. Although a limitation on the volume of available sera was responsible for fewer subjects being tested for IgA and IgM antibody, they were a subset of the group tested for IgG antibody.

To see if duration of colonization with MPA correlated with height of the titer to any of the ELISA antigens we compared duration of colonization and IgG or IgM titer for the 23 colonized patients whose duration of colonization was known. None of the

Table 1. *IgG-ELISA titers of patients with CF colonized with PA*

Patient	Yr colonized	113M WB	113M EXT	241M WB	241M EXT	256M WB	256M EXT	Na alg
1	8.4	20480*	10240	20480	640	5120	2560	80
2	>13.8†	40960	20480	40960	5120	20480	10240	320
3	7.3	40960	20480	81920	10240	10240	320	320
4	8.3	81920	40960	40960	40960	40960	10240	640
5	11.3	81920	81920	81920	40960	163840	163840	640
6	9.9	327680	163840	327680	40960	163840	327680	1280
7	2.7	327680	163840	81920	10240	81920	327680	1280
8	8.1	163840	163840	40960	10240	20480	163840	640
9	4.7	163840	163840	163840	20480	327680	81920	1280
10	>0.8	327680	327680	81920	40960	81920	81920	640
11	6.2	20480	10240	20480	2560	10240	10240	160
12	12.1	163840	163840	81920	20480	81920	81920	40
13	10.2	20480	81920	10240	5120	10240	10240	10
14	0.3	10240	10240	40960	640	5120	1280	0
15	8.6	163840	81920	40960	81920	163840	40960	640
16	10.8	327680	327680	327680	40960	327680	81920	640
17	6.6	5120	5120	5120	2560	5120	1280	‡
18	8.2	40960	40960	163840	40960	20480	20480	40
19	>4.8	163840	163840	163840	40960	163840	81920	1280
20	12.4	40960	163840	163840	10240	163840	163840	0
21	1.4	640	1280	2560	160	2560	320	40
22	4.2	163840	327680	163840	40960	163840	81920	1280
23	>8.2	81920	327680	81920	20480	40960	20480	160
24	>11.0	327680	327680	327680	5120	327680	163840	320
25	>10.3	81920	40960	81920	5120	81920	327680	80
26	>10.0	163840	163840	81920	5120	81920	327680	320
27	4.5	81920	81920	81920	10240	81920	40960	20
28	5.5	5120	5120	5120	640	5120	2560	20
29	>4.7	81920	163840	40960	5120	40960	40960	640
30	>10.7	40960	40960	40960	20480	40960	40960	2560
31	8.4	163840	163840	163840	40960	327680	163840	320
32	5.6	81920	163840	163840	40960	163840	163840	2560
Geometric mean		64552	65965	60491	10021	47666	31584	197

* Reciprocal of the endpoint titer. 327680 is the highest titer of the dilution series so all sera with this titer should be considered "greater than or equal to . . ." WB, whole bacteria; EXT, extract of MPA; Na alg, sodium alginate.

† Years of colonization preceded by ">" indicates patients who were colonized at the time of diagnosis or whose records prior to arrival at Y-NHH are unclear as to the earliest positive culture for MPA.

‡ Test not performed.

Table 2. *IgG-ELISA titers of patients with CF never colonized with PA**

Patient	113M WB	113M EXT	241M WB	241M EXT	256M WB	256M EXT	Na alg
1	5120	5120	5120	2560	640	640	320
2	5120	5120	5120	1280	5120	1280	0
3	2560	320	5120	40	2560	160	640
4	10240	2560	2560	640	2560	320	0
5	1280	640	640	160	1280	1280	80
6	5120	640	1280	160	1280	1280	160
7	40	40	40	10	80	80	0
8	10	0	10	0	40	10	0
Geometric mean	1076	370	761	110	761	293	15

* WB, whole bacteria; EXT, extract of MPA; Na alg, sodium alginate.

p values was significant ($p > 0.05$) in either a positive or negative direction.

It was noted that colonized patients with CF who had a high titer of antibody to one strain, extract, or to sodium alginate had a high titer to most or all of the antigens. Similarly, it appeared that relatively low titers to one antigen was linked to low titers to most or all of them (Table 1). In order to see whether these

trends were significant for the whole group the correlation coefficient between titers for each pair of antigens in Table 1 was calculated. The *p* values for these correlations were determined and are listed in Table 6. In general, the correlations were highly significant except that the correlation for antibody to the two other extracts (113M,241M) with the extract from 256M, was not significant ($p > 0.05$). Of note, the titer of antibody binding

Table 3. IgG-ELISA titers of normal volunteers*

Patient	113M WB	113M EXT	241M WB	241M EXT	256M WB	256M EXT	Na alg
1	640	640	2560	80	640	160	0
2	640	1280	320	80	1280	640	20
3	10240	40960	1280	80	1280	320	40
4	640	1280	640	20	640	160	20
5	1280	640	2560	160	640	320	640
6	640	80	1280	80	320	80	80
7	320	80	160	20	160	40	20
8	640	320	320	40	640	320	640
9	640	160	320	40	640	1280	320
10	640	160	640	20	640	160	80
Geometric mean	847	521	686	49	599	226	55

* WB, whole bacteria; EXT, extract of MPA; Na alg, sodium alginate.

Table 4. *p* values* of the differences between IgG-ELISA titers for three subject groups

ELISA antigen	Groups Compared		
	CF colonized vs CF uncolonized	CF colonized vs normals	CF uncolonized vs normals
113M whole bac- teria	<0.01	<0.01	NS†
113M extract	<0.01	<0.01	NS
241M whole bac- teria	<0.01	<0.01	NS
241M extract	<0.01	<0.01	NS
256M whole bac- teria	<0.02	<0.01	NS
256M extract	<0.02	<0.01	NS
Sodium alginate	0.05	<0.05	NS

* Determined by the two-tailed *t* statistic for two means.

† Not significant (*p* > 0.05).

to sodium alginate correlated with titers to each of the whole bacteria and extracts, even though antialginate titers were considerably lower.

When similar correlation determinations were calculated for the other two patient groups the strongly significant correlation between ELISA antibody titers to the different antigens, seen in the colonized patients with CF, was not present. In the group of CF patients not colonized with MPA, other than a correlation between 241M and 113M extracts (*p* < 0.05) there were no significant correlations. For the normal group, other than a correlation between titers of the homologous strains and extracts, there were no significant correlations.

When a similar evaluation was done for IgM-ELISA titers there was a significant correlation in the colonized CF group between titers against each of the MPA extracts and whole bacteria except for the following: the extract of 241M and 256 whole bacteria and between sodium alginate and each of the other antigens. For the other two patient groups there were no significant correlations between titers to any of the antigens.

The correlation between IgA-ELISA titers for different antigens was similar to the IgM-ELISA titers. For the colonized patients with CF the correlations between titers to each of the antigens was significant except between the extract of 241M and each of the other antigens and between sodium alginate and each of the other antigens.

Absorption experiment with whole MPA. Three sera, two from normal individuals and one from a patient with CF colonized with MPA, were absorbed with whole bacteria of strain 113. The IgG titer of the normal sera fell by two dilutions while the IgM titers fell from 1:40 and 1:80 to zero. The IgG titer of the patient with CF to whole 113M did not fall but the IgM titer fell by two

dilutions and the IgG and IgM titers to the extract of 113M fell by two and one dilution, respectively. All three sera had a drop of one or two dilutions of IgG and IgM to the whole bacteria and extracts of strains 241M and 256M.

ELISA on sera tested "blind" to the diagnosis. Eight sera and matching BAL specimens were collected by one of us (RBF) in the University of Iowa CF Center, coded, and sent to Connecticut for testing. The objective was to see if CF patients colonized with MPA could be distinguished from patients with other diagnoses using previously obtained serum ELISA titers of the Yale patients for discrimination. The four CF patients colonized with MPA (Table 7) were correctly identified prior to breaking the code based upon two hypothesized discriminating methods predicted to have value from observation of the data on the Yale sera. 1) IgG titers to the majority of antigens within 1 SD of the mean for the group and 2) a discrimination IgG titer of 1:10,000 for the whole bacteria and extracts distinguishing colonized CF patients from the others. As shown in Table 7, titers in excess of 1:10,000 were seen in all of the colonized CF patients from Iowa and only in one other subject (subject 1) to one antigen. Only three of 32 of the colonized Yale patients did not have a titer $\geq$ 1:10,000 and only one of 10 normal and one of eight noncolonized patients with CF had any titer $\geq$ 1:10,000.

ELISA on BAL fluids. IgG antibody in the BAL was seen in the colonized CF patients but there was some overlap with normals. Only patients 5 and 7 had substantially higher titers than the noncolonized subjects; however, a BAL IgG titer as high as 1:40 was seen in only one uninfected subject, to one antigen, (subject 2, to 113M whole bacteria) but a titer of $\geq$ 1:40 was seen in the BAL of each of the colonized CF patients.

Although IgG serum antibody was used to predict the diagnosis in the University of Iowa samples, the serum and BAL samples were also tested for IgM and IgA anti-MPA antibodies. Serum titers were similar to the Yale patients' titers shown in Table 5. IgM antibody in the BAL was absent by our ELISA in all subjects except patient 5 who had titers of 1:20 to 1:40 against the three whole organisms and their extracts. BAL IgA titers were similar to IgG titers but they were more clearly related to the colonization status. The normal subjects and the uncolonized CF patient had no IgA to any of the MPA antigens but all four colonized patients with CF had substantial IgA BAL titers (antiwhole 113M titers in subjects 5-8 were 1:256, 1:640, 1:160, and 1:10; titers against the other antigens were similar).

DISCUSSION

This study addresses the problem of understanding humoral immunity to MPA in patients with CF. We have demonstrated that chronically infected children and adults have high levels of broadly reactive anti-MPA antibodies, primarily of the IgG isotype. There is also substantial IgA but IgM titers are low. Only IgG titers were significantly higher in the CF patients who were

Table 5. Geometric mean titers of ELISA antibody to mucoid strains of PA in normal subjects, colonized and uncolonized patients with CF*

Isotype class of antibody	Subject group	N	ELISA antibody						Na alg
			113M WB	113M EXT	241M WB	241M EXT	256M WB	256M EXT	
IgG	CF-col	32	64552	65965	60491	10021	47666	31584	197
	CF-xcol	8	1076	370	761	110	761	293	15
	Normal	10	847	521	686	49	599	226	55
IgA	CF-col	28	5795	9507	3997	813	3997	6765	9
	CF-xcol	8	45	45	22	6	41	41	1
	Normal	8	48	107	31	16	58	31	2
IgM	CF-col	23	130	142	192	96	151	134	291
	CF-xcol	6	80	34	71	44	54	43	202
	Normal	6	59	107	108	108	88	59	359

* CF-col, patient with CF, colonized with mucoid PA; CF-xcol, patient with CF never colonized with PA; n, number of subjects tested; WB, whole bacteria; EXT, extract of MPA; Na alg, sodium alginate.

Table 6. Correlation of IgG-ELISA titers between the different ELISA antigens in the sera of patients with cystic fibrosis who were colonized with mucoid PA*

ELISA antigen	113M EXT	241M WB	241M EXT	256M WB	256M EXT	Na alg
113M WB	0.001†	0.001	0.05	0.001	0.001	0.001
113M EXT		0.001	0.05	0.001	NS‡	0.05
241M WB			0.05	0.001	0.01	0.05
241M EXT				0.01	NS	0.05
256M WB					0.05	0.01
256M EXT						0.01

* WB, whole bacteria; EXT, extract of MPA; Na alg, sodium alginate.

† *p* values of the correlation coefficient are less than these numbers (two-tailed test).

‡ Not significant (*p* > 0.05).

Table 7. IgG ELISA on paired sera and BAL (blind to diagnosis)*

Patient	Specimen	113M WB	113M EXT	241M WB	241M EXT	256M WB	256M EXT	Diagnosis
1	Serum	5120	2560	1280	40	10240	640	Normal
	BAL	10	0	0	0	0	0	
2	Serum	320	320	320	80	160	640	Normal
	BAL	40	20	0	0	0	0	
3	Serum	320	640	320	80	320	640	Normal
	BAL	0	0	10	0	0	0	
4	Serum	1280	2560	640	640	1280	640	CF (uncolonized)
	BAL	10	20	0	0	20	0	
5	Serum	40960	40960	20480	20480	40960	20480	CF (colonized)
	BAL	1280	1280	1280	160	1280	320	
6	Serum	20480	5120	20480	1280	10240	10240	CF (colonized)
	BAL	80	40	80	20	80	40	
7	Serum	5120	2560	20480	5120	5120	5120	CF (colonized)
	BAL	160	40	1280	80	160	80	
8	Serum	20480	20480	10240	2560	10240	10240	CF (colonized)
	BAL	10	40	0	0	10	40	

* WB, whole bacteria; EXT, extract.

colonized with MPA, compared with uncolonized patients and normal young adults. We have also shown that colonized patients have antibody to MPA in the bronchial-alveolar fluid. Since CF patients who were never known to have been infected with MPA

had significantly less antibody than infected patients and had titers not significantly greater than normal young adults who are not susceptible to infection with MPA, the ELISA measures a specific MPA-induced IgG immune response. This assumption

was tested on a small collection of specimens from the University of Iowa and the four patients with CF who were colonized with MPA were identified of the eight subjects on the basis of the serum IgG titers against MPA. While the majority of these patients clearly fell in one group or the other based upon review of the data from the patients at Yale there were still some individual patients whose titers were in between. Thus from the set of patients from Iowa, subjects 1 and 7 (Table 7) had titers which were not very different and three of the colonized patients from Yale had serum titers which were not higher than many of the normal subjects.

Pier *et al.* (8), Speert *et al.* (9), and Bryan *et al.* (10) have previously shown that infected patients with CF have high titers of antibody to the mucoid exopolysaccharide of MPA but they tested purified extracts from one strain. Speert *et al.* (9) also showed that these patients have serum immunoreactivity to sodium alginate but in contrast to our study their results showed that the titer correlated with length of chronic infection. Most of our patients had a long duration of colonization and it is quite possible that antibody rises to a certain titer and then plateaus. A prospective study with samples taken at short intervals might give different results. Using a statistical analysis we have demonstrated that the titer of immunoreactivity to sodium alginate is correlated with the titer to the whole organism and an extract of three strains, each with a different immunotype (primarily lipopolysaccharide) antigen. Absorption of sera with one of the strains reduced the IgG and IgM titer to the whole cells and extracts of the other two. While the titers were not reduced to zero, it appears that most of the anti-MPA immunoreactivity in the normal sera and serum from an infected subject was directed toward a shared antigen or antigens. These results are consistent with the hypothesis that while chronically infected patients with CF develop antibody to a lipopolysaccharide antigen (18) a major part of the antibody response is to a common antigenic determinant or determinants which is not the lipopolysaccharide antigen. If the antibody response was to the immunotype or lipopolysaccharide antigen, infected patients would have antibody measurable to one immunotype but not the others and absorption should only remove antibody to strains having the same immunotype as the absorption strain. Such a response is seen with infections due to nonmucoid strains of PA (19).

The finding of anti-*Pseudomonas* antibody in the BAL, primarily of the IgG and IgA isotype, is intriguing. It was demonstrated by Schiøtz *et al.* (20), using an immunofluorescence assay, that colonized patients with CF have anti-*Pseudomonas* antibody in the sputum, while uncolonized patients do not. The data presented herein on a small number of patients show that this is also true of the BAL and the titers appear to parallel the serum antibody titer for IgG, IgA, and IgM. The *in vivo* function of this antibody is unknown since MPA are generally never completely cleared from the lungs. Among the factors that may be responsible for this failure are the proposed defective function of antibody to MPA in patients with CF (18), leukocyte and *Pseudomonas* IgG protease in CF sputum (21), and relative resistance of MPA to binding opsonophagocytic antibody (12).

The results of this study may have implications for research efforts aimed at the development of an anti-MPA vaccine. *Pseudomonas* vaccines have been effective for patients with burns in which an LPS extract is used and multivalent vaccines are required for protection (22), but no human trials of an MPA or alginate vaccine have, as yet, been reported. This study does not address the question of whether antibody to a shared antigen such as the alginate exopolysaccharide would be protective against the effects of infection or if it would prevent colonization,

but these data do suggest that a response to such antigens exists in chronically infected patients. One interesting finding of this study in terms of possible vaccine strategy is that anti-MPA antibody is found in the lung, albeit in low titer relative to the serum. It is not clear if this antibody represents exuded serum antibody or endogenous pulmonary production of antibody. The presence of this antibody, however, demonstrates the potential for local airway immunity in individuals who possess systemic humoral immunity.

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