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Prospective Study of Serum Antibodies to *Pseudomonas aeruginosa* Exoproteins in Cystic Fibrosis

ANNIKA ERICSSON HOLLSING,[1,2]* MARTA GRANSTRÖM,[2] MICHAEL L. VASIL,[3] BENGT WRETLIND,[4] AND BIRGITTA STRANDVIK[1]

Department of Pediatrics, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge,[1] Department of Vaccine Production, National Bacteriological Laboratory, S-105 21 Stockholm,[2] and Department of Clinical Bacteriology, Danderyd Hospital, S-182 88 Danderyd,[4] Sweden, and Department of Microbiology and Immunology, University of Colorado Medical School, Denver, Colorado 80262[3]

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Serum immunoglobulin G to four purified antigens from *Pseudomonas aeruginosa*, phospholipase C, alkaline protease, exotoxin A, and elastase, were determined in 62 patients with cystic fibrosis by enzyme-linked immunosorbent assay. The patients were followed for 12 to 24 months in a prospective study. Increased titers, i.e., titers more than 2 standard deviations above those of normal controls, were exclusively found in patients chronically colonized with *P. aeruginosa* and not in patients harboring only *Staphylococcus aureus*. The frequencies of elevated titers of antibody to the different antigens varied from 100% (phospholipase C) to 58% (alkaline protease and exotoxin A) to 15% (elastase) in the chronically colonized patients. Mean serum titer levels, expressed as multiples of the age-correlated upper normal limit (=1), were significantly higher to phospholipase C in patients with dual colonization with *P. aeruginosa* and *S. aureus* than in those colonized only with *P. aeruginosa* ($P < 0.001$). Conversely, the other three antigens showed significantly higher serum antibody titer levels in patients harboring only *P. aeruginosa* ($P < 0.001$). In five patients who became colonized with *P. aeruginosa* during the study period, serum antibodies to phospholipase C and exotoxin A increased first. Exceptions to the general pattern of antibody responses were found in three patients chronically colonized with *Escherichia coli*. They showed a delayed enhancement of anti-phospholipase C titers after the chronic *P. aeruginosa* colonization. Serum titers were not influenced by exacerbations of pulmonary infection or by antimicrobial therapy. The determination of titers of serum antibody to phospholipase C seems to be a valuable indicator of a chronic colonization with *P. aeruginosa*. The results further suggest that bacterial metabolism and interactions may influence the antibody response.

The chronic respiratory tract infection, the hallmark of cystic fibrosis (CF), is still the major factor in determining the severity of illness and mortality (22). The predominating pathogens isolated from sputum are *Staphylococcus aureus* and *Pseudomonas aeruginosa* (12). *S. aureus* is usually the initial pathogen, succeeded by *P. aeruginosa* after different lengths of time (22), for which the prognosis is commonly considered poor (16, 25, 26). It seems as though frequent flare ups of active infection superimpose and complicate the chronic colonization. Although more than short-term eradication of *P. aeruginosa* is seldom achieved, exacerbations can be brought into remissions by antibiotic treatment (16, 24, 28). An important task is therefore to identify early signs of infection and the optimal time of starting antimicrobial therapy (21, 24).

Serological assays, determining the levels of antibodies against *S. aureus* products, have been used to follow the course of different staphylococcal infections (33). In patients with CF, these assays have been found valuable in monitoring treatment (6; A. Ericsson Hollsing, M. Granström, and B. Strandvik, Arch. Dis. Child., in press). Serum antibody levels to exoproteins of *P. aeruginosa* (exotoxin A, elastase, and alkaline protease) have been found to be increased in CF (4, 13, 15, 17, 18). A correlation between chronic colonization with *P. aeruginosa* and elevated titers of serum antibody to phospholipase C and alkaline protease has been reported (9). A possible relation between clinical signs of exacerbations and increased titers of serum antibody to exotoxin A and elastase was suggested (9). To elucidate the clinical value of the determination of these antibodies in relation to signs of infection and antimicrobial therapy, a prospective study was performed in patients with CF.

MATERIALS AND METHODS

Patients. Sixty-two patients with CF, aged 4 months to 32 years (mean and medium, 12.5 years), were studied. The diagnosis was based on a pathological sweat test (chloride, >80 mmol/liter) and typical pulmonary symptoms. All patients had normal height and weight and all but two had pancreatic insufficiency. No patient was given corticosteroids. All patients were routinely seen at monthly check-ups, and sputum cultures were done. Growth of *Pseudomonas cepacia* was never found during the study period. The results of sputum cultures during the 6 months preceding the study was the basis for classification of the patients into seven groups.

(i) Group P. Eight patients, aged 16 to 32 years (mean, 20.2 years), were chronically colonized with *P. aeruginosa*; i.e., *P. aeruginosa* was consistently present in sputum. *S. aureus* was not found.

(ii) Group ISP. Six patients, aged 6 to 19 years (mean, 12.5 years), were chronically colonized with *P. aeruginosa*, and *S. aureus* was intermittently found in sputum.

(iii) Group SP. Twelve patients, aged 10 to 26 years (mean, 15.7 years), were chronically colonized with both *P. aeruginosa* and *S. aureus*; both bacteria were consistently found in sputum cultures.

* Corresponding author.

(iv) **Group SIP.** Four patients, aged 2 to 20 years (mean, 10.8 years), were chronically colonized with *S. aureus* and also had intermittent growth of *P. aeruginosa* in sputum.

(v) **Group S.** Fifteen patients, aged 6 to 27 years (mean, 15.3 years), were chronically colonized with *S. aureus*. Nine patients occasionally showed additional growth in sputum of *Klebsiella* species ($n = 2$), *Haemophilus influenzae* ($n = 8$), and/or *Pseudomonas maltophilia* ($n = 1$).

(vi) **Group E.** Three patients, aged 9, 10, and 24 years, were chronically colonized with *Escherichia coli* according to sputum cultures. All had additional chronic colonization with *P. aeruginosa*, and two also were colonized with *S. aureus*.

(vii) **Group NP.** Fourteen patients, aged 4 months to 5 years (mean, 2.2 years), were too young to present sputum. Nasopharyngeal cultures showed occasional growth of *H. influenzae*, *S. aureus*, and *Haemophilus parainfluenzae* but never *P. aeruginosa*.

Clinical study. The study was planned for a period of 1 year. Sampling was continued for another year in most patients (44 of 62, 71%). In addition, earlier samples were available in 25 patients (40%), resulting in an observation period in these patients of 24 to 94 months. The bacterial colonization changed in five patients during this period from absence of or intermittent to chronic colonization with *P. aeruginosa*. The samples from these patients are reported in the relevant group at the time of the examinations.

The clinical score was recorded according to Shwachman and Kulczycki (27) excluding X-ray studies; thus, a maximum score of 75 indicated an excellent clinical condition. The clinical score ranged from 30 to 75 (mean, 60; median, 63) at the beginning of the study. The pulmonary X ray was evaluated by a modified Norman-Cris-pin score (19). The mean X-ray score was 3 (median, 3; range, 0 to 12), a value of 0 indicating a normal picture and a maximum value of 16 units indicating severe disease. One-second forced expiratory volume ($FEV_{1.0}$) was determined at least once a year in patients older than 6 years and is expressed as a percentage of predicted values for age (5). A mean value of 67% (median, 65; range, 20 to 111) was found at the first investigation during the study.

Clinical symptoms of infection, such as change in volume, appearance, or color of sputum, increased respiratory rate or dyspnea, progressive physical findings on chest auscultation, increased cough, decreased appetite, and or loss of weight and deterioration of standard biochemical tests, were used as indications for antimicrobial treatment. The presence of more than two of these criteria was also the basis for classifying the patients as "infected" or "noninfected." The patients seldom had elevated erythrocyte sedimentation rate (ESR) or leukocyte count (WBC) and very rarely had temperature elevations; thus, the clinical signs of infection were discrete at the start of antibiotic therapy.

During the 24-month period, 100 intravenous antibiotic treatments were given to the patients on an in-patient basis. Antibody titers were measured in blood samples at the start and end of antibiotic therapy, the average interval being 13 days (range, 7 to 29 days). Twenty-one treatments were given to nine patients harboring *P. aeruginosa* alone and 50 courses were given to patients with additional chronic ($n = 31$) or intermittent ($n = 19$) growth of *S. aureus* in sputum. Two of the three patients in group E had five antimicrobial courses during the study period. The remaining treatments were given to patients not chronically colonized with *P. aeruginosa* (12 courses each to the patients in groups SIP and S [not included in the results]). The type of chemotherapy chosen was individualized depending on the resistance pattern of the bacteria (7, 29) and most often (63%) was cephalosporins in combination with aminoglycosides.

Analysis of sputum. The standard procedure of handling sputum samples at Huddinge University Hospital was used (8). Briefly, the sputum samples were placed in an ordinary tea strainer and washed with tap water. Thereafter, the samples were Gram stained and examined microscopically to obtain the ratio of leukocytes/squamous epithelial cells. Smears with leukocytes/epithelial cell ratios of >5 were classified as purulent and representative of sputum. Sputum cultures were performed in a semiquantitative manner. The specimens were homogenized with dithiothreitol (Sputolysine; Calbiochem-Behring), diluted 1/100, and spread with disposable standardized plastic loops containing 10 μl onto plates of the following six media: (i) blood agar with 5% horse blood for isolation of *S. aureus*; (ii) pseudomonas isolation agar (Difco Laboratories) with a factor V disk placed on the agar for the selective isolation of *Pseudomonas* species; (iii) CLED agar (made in house) for isolation of other gram-negative bacilli; (iv) blood agar with 5% sheep blood and gentian violet 0.01% for the selective isolation of beta-hemolytic streptococci and pneumococci; (v) hemin-bacitracin agar for *Haemophilus* species; (vi) hematin agar for identification of all bacteria. Plates i to iii were incubated at 37°C aerobically for 48 h, plates iv and v were incubated anaerobically for 24 h, and plate vi was incubated at 37°C in 5% carbon dioxide for 48 h. The isolated bacteria were identified according to standard techniques. Normal pharyngeal flora cultured in sputum were not recorded, whereas all isolates in the bronchial secretion were. Antibiotic susceptibility was determined by the ICS method (7, 29) with paper disks (AB Biodisk, Sweden).

Sera. Capillary or venous blood samples were drawn from all patients at least once a year. In most patients, samples were also obtained before and after periods of antibiotic treatment. Sera were frozen at −20°C until analyzed. All sera from each patient were analyzed in one series.

Antigens. *Pseudomonas* elastase and *Pseudomonas* alkaline protease were purchased from Nagase Biochemicals, Fukuchiyama, Kyoto, Japan. Exotoxin A was bought from the Swiss Serum and Vaccine Institute, Bern, Switzerland. The purification of phospholipase C from a mutant of strain PAO1 has been described earlier (1). Purity was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; phospholipase C gave one band, and elastase, alkaline protease, and exotoxin A gave one major band but also a few minor ones, which may be due to contaminants or degradation products.

ELISA. The methodological details of the enzyme-linked immunosorbent assay (ELISA) have been described (9, 10). Positive and negative controls (three patients and three healthy individuals) were included in each test series to minimize the day-to-day variations. The coefficients of variations for interassay determinations were 10 to 15%, and for intraassay determinations they were 5 to 10%. Increased titers were defined in relation to antibody titers in the normal population of 196 healthy controls of different ages (9). As the limits of the upper normal values of ELISA titers were age dependent, an increased titer in one age group could represent a normal value in another age group. Due to the long observation period for some children, different cutoff levels had to be applied for samples from different times in the same patient. Therefore, ELISA titers are presented as relative titers, i.e., multiples of the upper limit of normal values (mean ± 2 standard deviations), which was set at 1.

TABLE 1. Relative serum antibody titer levels (mean ± standard deviation) in patients clinically infected (i) and noninfected (ni)[a]

Antibody to:	Relative serum titer (range)										
	Group P		Group ISP		Group SP		Group SIP		Group S		Group NP, i + ni (n = 16)
	i (n = 35)	ni (n = 51)	i (n = 31)	ni (n = 46)	i (n = 56)	ni (n = 88)	i (n = 18)	ni (n = 25)	i (n = 41)	ni (n = 66)	
Phospholipase C	2.7 ± 0.4 (1.7–3.3)	2.7 ± 0.3 (1.7–3.2)	3.3 ± 0.6 (2.1–4.4)	3.1 ± 0.7 (1.8–5.1)	3.4 ± 1.2 (1.0–5.7)	3.3 ± 1.2 (1.0–5.7)	0.4 ± 0.3 (0.1–1.0)	0.5 ± 0.3 (0.1–1.0)	0.4 ± 0.2 (0.1–1.0)	0.4 ± 0.2 (0.1–1.0)	0.3 ± 0.2 (0.1–0.8)
Alkaline protease	2.0 ± 1.0 (0.4–4.0)	2.0 ± 1.2 (0.5–4.0)	1.8 ± 0.4 (0.4–2.3)	1.7 ± 0.5 (0.5–2.6)	1.3 ± 0.6 (0.1–2.5)	1.2 ± 0.6 (0.2–2.9)	0.2 ± 0.1 (0.1–0.5)	0.3 ± 0.8 (0.1–0.5)	0.2 ± 0.2 (0.1–0.7)	0.3 ± 0.1 (0.04–0.6)	0.3 ± 0.2 (0.1–0.7)
Exotoxin A	3.0 ± 2.0 (0.6–6.7)	3.2 ± 2.2 (0.7–6.7)	1.7 ± 1.4 (0.5–5.1)	1.7 ± 1.3 (0.4–5.1)	1.7 ± 1.2 (0.3–5.9)	1.7 ± 1.3 (0.1–5.7)	0.4 ± 0.3 (0.04–1.0)	0.4 ± 0.2 (0.1–0.9)	0.4 ± 0.2 (0.1–0.9)	0.4 ± 0.2 (0.1–1.0)	0.2 ± 0.2 (0.1–0.7)
Elastase	1.8 ± 1.8 (0.1–5.9)	1.6 ± 1.8 (0.1–6.7)	0.9 ± 0.9 (0.1–2.6)	1.0 ± 0.9 (0.1–2.7)	0.5 ± 0.5 (0.1–2.2)	0.4 ± 0.4 (0.1–1.9)	0.1 ± 0.1 (0.0–0.5)	0.1 ± 0.1 (0.04–0.3)	0.1 ± 0.03 (0.1–0.2)	0.1 ± 0.1 (0.0–0.2)	0.1 ± 0.1 (0.0–0.6)

[a] Patients are grouped according to chronic bactrial colonization: P = *P. aeruginosa*; ISP = *P. aeruginosa* plus intermittent *S. aureus*; SP = *P. aeruginosa* plus *S. aureus*; SIP = *S. aureus* plus intermittent *P. aeruginosa*; S = *S. aureus*; NP = nasopharyngeal cultures. For further explanation, see Materials and Methods.

Values of >1 were thus increased. The mean relative titers in the healthy population ranged from 0.3 to 0.8 in the different age groups.

Statistics. The chi-square test with Yates correction, linear regression, the Mann-Whitney U test for differences between means (MW), and the Wilcoxon matched-pairs signed ranks test were used.

RESULTS

Clinical parameters. Over the 2-year study period, there was no change in average values of clinical score, X-ray score, or $FEV_{1.0}$ in the whole group of patients, indicating rather stable disease. The patients classified as noninfected had a normal average WBC of 6,800 per μl (n = 159; range, 2,000 to 13,600) and normal ESR of 13 mm (n = 142; range, 2 to 47). Most patients showed only slight symptoms of infection when classified as infected (e.g., loss of appetite, increased coughing), illustrated by a mean WBC showing a moderate increase to 9,500 per μl (n = 145; range, 2,400 to 20,500) and an ESR to 21 mm (n = 132; range, 2 to 98).

The 76 intravenous antibiotic treatments resulted in clinical improvements with less sputum production, less coughing, and usually an increase in body weight. A decrease in WBC from 9,800 (n = 68; range, 2,400 to 19,100) at pretreatment to 7,100 (range, 2,100 to 14,100) per μl post-treatment was seen ($P < 0.001$, Wilcoxon). The corresponding ESR was not influenced, the average value being 18 mm (n = 55; range, 2 to 61) before and 16 mm after (range, 2 to 40, not

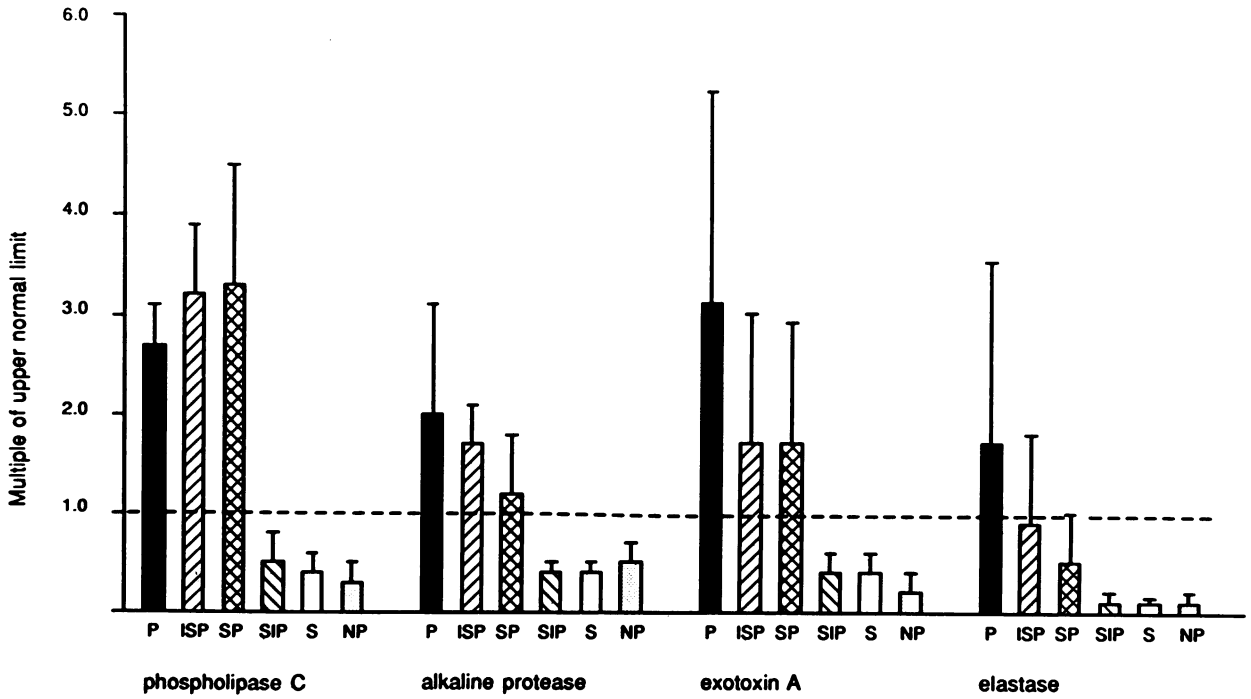

FIG. 1. Relative titers of serum antibody to *P. aeruginosa* exoproteins, phospholipase C, alkaline protease, exotoxin A, and elastase, in the various patient groups. Values shown are the means ± standard deviation for all samples in each bacterial colonization group. The upper normal limit is 1.0. See text for group designations.

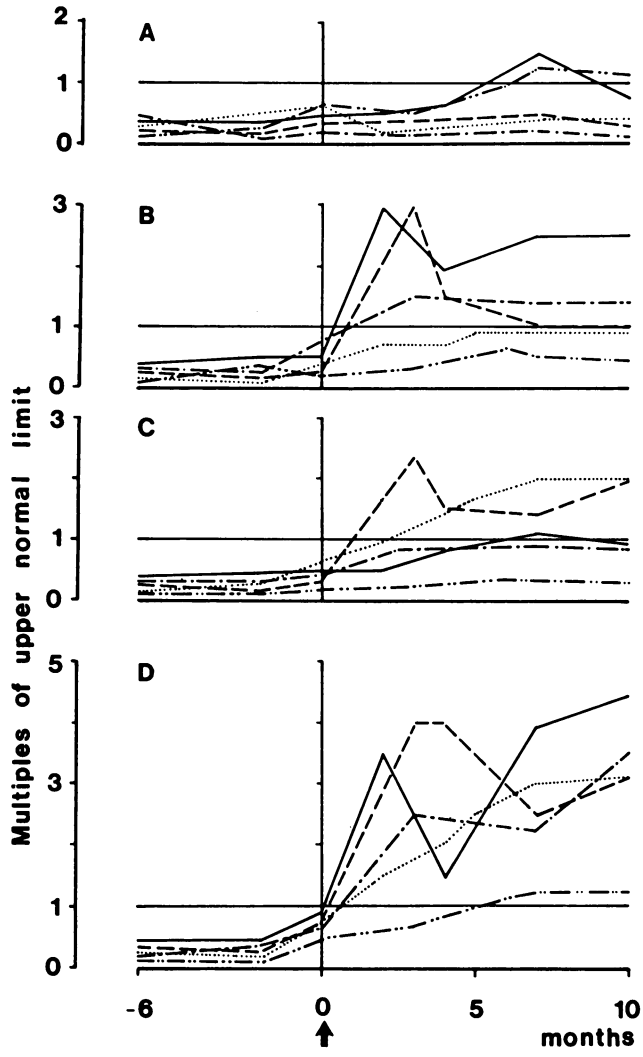

FIG. 2. Relative titers of serum antibody to *P. aeruginosa* exoproteins, elastase (A), exotoxin A (B), alkaline protease (C), and phospholipase C (D), in five patients chronically colonized with *S. aureus* and intermittently colonized with *P. aeruginosa*. Arrow indicates establishment of chronic *P. aeruginosa* colonization. The patients are designated by different types of lines. The time for intermittent *P. aeruginosa* colonization varies between 6 months and >3 years.

significant, Wilcoxon) treatment. $FEV_{1.0}$ showed significant improvement, increasing from a mean of 52% (n = 46; range, 13 to 99) to 63% (range, 18 to 110; $P < 0.001$, Wilcoxon).

Serum antibodies to the four antigens. ELISA immunoglobulin G titers of antibody to phospholipase C, alkaline protease, exotoxin A, and elastase were determined in 515 serum samples from the 62 patients. The mean relative titers in the various groups are shown in Table 1. Regardless of bacterial colonization, there were no significant differences in serum titer levels when samples were drawn from clinically infected or noninfected patients. Figure 1 shows the mean values of relative serum titer levels in the total material (except group E) grouped according to bacterial colonization of the airways. Increased serum titers, i.e., >1, were solely found in patients chronically colonized with *P. aeruginosa* (groups P, ISP, and SP), while patients in groups SIP, S, and NP (not colonized with *P. aeruginosa*) all had titers within the normal range ($P < 0.001$, MW). The five patients acquiring chronic *P. aeruginosa* colonization during the observation period increased their serum anti-phospholipase C titers at an average of 3 months (range, 2 to 6 months) after the colonization was chronically established. The other *Pseudomonas* antibodies were not consistently elevated and often appeared later: anti-exotoxin A after 2 to 15 months, anti-alkaline protease after 3 to 28 months, and anti-elastase after 6 to 28 months (Fig. 2).

Antibodies to phospholipase C. All patients chronically colonized with *P. aeruginosa* had elevated titers of serum antibody to phospholipase C. The mean value was significantly higher in patients with dual colonization of *P. aeruginosa* and *S. aureus* than in those solely harboring *P. aeruginosa* ($P < 0.001$, MW). There was no significant difference in antibody titer levels between patients colonized with mucoid versus nonmucoid strains (2.8 versus 2.5; not significant, MW).

Antibodies to alkaline protease. The mean serum titer level was significantly higher in patients harboring only *P. aeruginosa* than in those with *P. aeruginosa* and *S. aureus* ($P < 0.001$, MW). Contrary to the antibody response to phospholipase C, which was elevated in 100% of patients with chronic *P. aeruginosa* colonization, 11 patients (42%) had normal relative titers of antibody to alkaline protease, not related to dual bacterial colonization. There was significantly higher mean serum titer levels in patients harboring mucoid versus nonmucoid strains (1.6 versus 0.6; $P < 0.01$, MW).

Antibodies to exotoxin A. Increased serum titers of antibodies against exotoxin A were found in 58% of patients chronically colonized with *P. aeruginosa*. This was more common in samples from patients with only *P. aeruginosa* than in those from patients with dual colonization (86 versus 64%; $P < 0.001$, chi square). Mucoid strains seemed to induce a more pronounced antibody response than nonmucoid strains (mean values, 3.4 and 1.8, respectively; $P < 0.05$, MW).

Antibodies to elastase. Fifteen percent of the patients chronically colonized with *P. aeruginosa* had elevated titers of serum antibody to anti-elastase. Patients harboring *P. aeruginosa* only had significantly higher mean values than those yielding *S. aureus* in addition (1.69 versus 0.45; $P < 0.001$, MW). Patients with mucoid strains had significantly higher serum antibody titers than those harboring only nonmucoid strains (means, 1.9 versus 0.4; $P < 0.05$, MW).

Correlation of serum antibodies to clinical parameters. Serum antibodies to exotoxin A showed a weak, inverse correlation to clinical score ($r = -0.29$; $P < 0.05$, linear regression). No correlations were found between the antibody responses to the four antigens of *P. aeruginosa* and the $FEV_{1.0}$ or X-ray score. Age of the patient correlated inversely to the serum level of anti-phospholipase C ($r = -0.40$; $P < 0.01$, linear regression). Anti-alkaline protease titers in serum correlated to the duration of chronic *P. aeruginosa* colonization ($r = 0.56$; $P < 0.001$, linear regression).

Relation between serum antibodies and antimicrobial treatment. There was no significant decrease in any antibody titer level in serum after antimicrobial treatment except in a few patients (6 of 23 courses in five patients). No consistent pattern of bacterial colonization, choice of antimicrobial agent, length of colonization, age of patient, clinical or X-ray score, or $FEV_{1.0}$ could be found in these courses compared to other courses in the same patients or between these patients and the others.

Serum antibodies in chronic *E. coli* colonization. Excep-

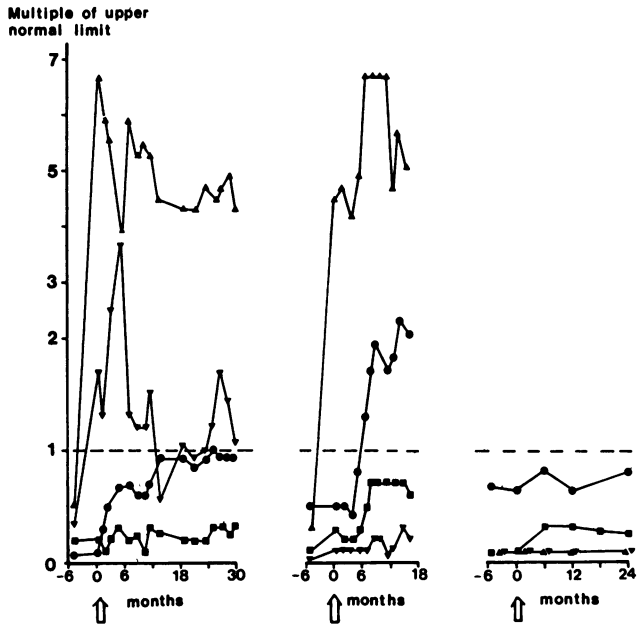

FIG. 3. Relative titers of serum antibody to *P. aeruginosa* exoproteins, phospholipase C (●), alkaline protease (■), exotoxin A (▲), and elastase (▼), in three patients chronically colonized with *E. coli*. Arrows indicate establishment of chronic *P. aeruginosa* colonization.

tions to the general pattern of serum antibody responses in patients chronically colonized with *P. aeruginosa* were seen in the three patients with chronic colonization with *E. coli*. These patients could be followed when they displayed, in addition, chronic colonization with *P. aeruginosa*. Two of the patients increased their serum titers of anti-exotoxin A to enhanced levels, but the anti-phospholipase C serum titers increased much later; in one patient the latter titers were still normal 30 months after the chronic *P. aeruginosa* colonization (Fig. 3). The third patient had normal titers of serum antibody to all antigens after 2 years. These three patients did not differ in other respects from the patients chronically colonized with *P. aeruginosa*.

DISCUSSION

Enhanced relative titers of serum antibody to *Pseudomonas* exoproteins were exclusively found in patients chronically colonized with *P. aeruginosa*. Patients not or only intermittently colonized with *P. aeruginosa* in addition to chronic *S. aureus* colonization all had relative serum titers within the normal range. This is in accordance with other studies with precipitins or nonpurified antibodies to *P. aeruginosa* (3, 13). The titers of serum antibody to phospholipase C showed 100% sensitivity since all patients and all samples from patients harboring *P. aeruginosa* chronically showed elevated titers. The *Pseudomonas* antibodies developed differently in the five patients who could be followed during the establishment of the chronic colonization. The phospholipase C antibodies developed first, at an average of 3 months after colonization, and the other pseudomonad antibodies developed later, in accordance with the findings of Döring and Høiby (4). It therefore seems that the antibody determination of phospholipase C could be used as a marker of chronic *P. aeruginosa* colonization, except in those patients who chronically harbor *E. coli*.

Patients with mucoid strains of *P. aeruginosa* had significantly higher relative titers of serum antibody to alkaline protease, exotoxin A, and elastase than those harboring only nonmucoid strains. Høiby also found that mucoid strains were associated with a pronounced antibody response, in contrast to nonmucoid strains (12). Studies of antigen expression in mucoid and nonmucoid *Pseudomonas* strains in vitro have shown other results (11, 14, 26), indicating that the complexity of in vivo situations may interfere with the antigen-antibody response. This is illustrated in the patients chronically colonized with *E. coli*. In two patients, 24 to 30 months of additive *P. aeruginosa* colonization did not result in the expected increase in anti-phospholipase C titers in serum. Two of these three patients with dual colonization with *E. coli* and *P. aeruginosa* had elevated titers of antibody to exotoxin A before the anti-phospholipase C titers increased. Exotoxin A, alkaline protease, and elastase are regulated by the iron content in the environment. The formation of these exoproteins is increased during iron-limited growth (2, 31, 32). In vivo, *P. aeruginosa* and *E. coli* probably compete with each other for access to iron, and *E. coli* will consume iron which may limit the amount of iron available to *P. aeruginosa*, thus stimulating the production of iron-regulated extracellular products. On the other hand, phospholipase C and alkaline phosphatase are regulated by the inorganic phosphate content in the environment (31). The function of these enzymes is to release bound phosphate, which can be used by the bacterium. It is possible that *E. coli*, which also produces a phosphate-regulated alkaline phosphatase (30), will make inorganic phosphate available to *P. aeruginosa*, which in turn will cause a decrease in the formation of phospholipase C.

Patients with only *P. aeruginosa* colonization had significantly higher relative titers of serum antibody to alkaline protease (means, 1.99 versus 1.39), exotoxin A (3.11 versus 1.69), and elastase (1.69 versus 0.62) compared to patients with a dual colonization with *P. aeruginosa* and *S. aureus* ($P < 0.001$, MW). The result was the reverse for serum antibodies to phospholipase C; patients chronically harboring both *P. aeruginosa* and *S. aureus* had higher anti-phospholipase C levels (means, 3.28 versus 2.69; $P < 0.001$, MW). In a study of *S. aureus* products, anti-teichoic acid serum titer levels were significantly higher in patients with dual colonization than in patients harboring only *S. aureus* (Ericsson Hollsing et al., in press). If the high serum antibody levels reflect a more serious infection, these two findings could have clinical relevance for the often reported observation that patients deteriorate rapidly once *P. aeruginosa* colonization is established (12, 22, 25). It may imply that special efforts should be made to eradicate *S. aureus* when *P. aeruginosa* appears. In our experience the colonizing pattern is seldom abruptly shifted from *S. aureus* to *P. aeruginosa* and both pathogens tend to be present for varying periods of time. Very high serum titers against phospholipase C might suggest such dual colonization.

Our finding of a correlation between length of *P. aeruginosa* colonization and relative titers of serum antibody to alkaline protease is in agreement with the results of others (4, 13). There was, however, no increase in these serum titers during the follow-up period of 2 to 5 years in individual patients in our study, suggesting a very slow enhancement of serum titers. This is contrary to whole-cell precipitins, which increased each year (4, 12, 13). The relative serum anti-phospholipase C titers, which seem to be the main colonization marker, did not increase with length of chronic colonization, but decreased with increasing age of patients.

Earlier studies of antibodies to pseudomonad exoproteins (4, 13, 17, 18), unpurified *Pseudomonas* cell surface antigens (3), and precipitins (4) in serum found correlations with clinical score. In this study, only serum exotoxin A antibody titers showed a similar tendency. Patients with normal relative serum antibody titers and chronically colonized with *P. aeruginosa* were not found to be different in any clinical parameter from those with increased levels. Also, in the patients (n = 8) with extremely high titers (>3), no significant difference could be found in clinical or X-ray score, lung function ($FEV_{1.0}$), age, length or type of colonization (P or SP), or type of strain (mucoid or nonmucoid) compared to patients with normal titers. This is not in agreement with the afore-mentioned studies (3, 4, 13, 17, 18) and might reflect differences in co-colonization, properties of the *Pseudomonas* strains (20, 23, 31), or biochemical or immunological factors.

There was no difference between relative titers of serum antibody to any of the examined antigens in patients clinically infected and not infected (Table 1). This observation differs from the findings of others, who could correlate active pulmonary infection and high titers of serum antibody to alkaline protease and exotoxin A (15) or unpurified antigens (3). This difference from other studies (3, 15) might be methodological or due to our classifying patients with very mild symptoms as infected. With a few exceptions, antimicrobial treatment in our patients did not result in a decrease in titers of antibody to any of the *Pseudomonas* exoproteins despite significant clinical improvement and normalization of standard blood parameters of infection. This is in agreement with other studies (3, 4, 13) but contrary to our findings for antibodies against *S. aureus* products (Ericsson Hollsing et al., in press). Increased relative titers of serum antibody to both teichoic acid and alpha-toxin reflected exacerbations of the chronic pulmonary infection significantly more sensitively than WBC and ESR, and the serum titers could be decreased subsequent to antimicrobial treatment. The differences in antibody patterns in response to therapy cannot be explained at present.

In conclusion, determination of immunoglobulin G in serum against *Pseudomonas* phospholipase C seems to be an excellent serological measurement (100%) of chronic *P. aeruginosa* colonization. This might be especially valuable if representative sputum samples are difficult to obtain. The serum antibodies to alkaline protease or exotoxin A could be used to confirm chronic colonization with *P. aeruginosa* in about 60% of the patients but not to reveal mild acute exacerbations of pulmonary infection. The low percentage of our patients showing serum elastase antibodies suggests a low sensitivity of this antibody for the diagnosis of infection and may reflect our policy of treating patients with early signs of infection. It does not exclude that anti-elastase might be an indicator of more fulminant infections. The results suggest that bacterial metabolism and interactions probably can influence the antibody response directly or via immunological factors. The pathogenicity and virulence of *P. aeruginosa* and its exoproteins in CF are obviously multifactorial and require further studies.

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