Altered Antibody Isotype in Cystic Fibrosis: Impaired Natural Antibody Response to Polysaccharide Antigens¹

RICHARD B. MOSS, YAO-PI HSU, PETER H. VAN EEDE, ALFRED M. VAN LEEUWEN,
NORMAN J. LEWISTON, AND GERDA DE LANGE

*Ross Mosier Laboratory for Cystic Fibrosis Research, Children's Hospital-Stanford, Department of Pediatrics,
Stanford University Medical School, Palo Alto, CA 94304 and Department of Immunogenetics, Central
Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam [P.H.V.E., A.M.V.L., G.D.L.]*

ABSTRACT. Patients with cystic fibrosis (CF) have impaired natural (preinfection) IgG2 antibody responses to *Pseudomonas aeruginosa* lipopolysaccharide. To investigate the basis for this defect, we measured natural IgG and IgG1-4 antibody levels to *Haemophilus influenzae* type b polyribophosphate (PRP) and tetanus toxoid by enzyme-linked immunosorbent assay in 24 adult CF patients and 20 normal controls. Immunoglobulin heavy- and light-chain allotypes were determined on 146 Caucasian CF patients and 96 controls. The tetanus toxoid-specific IgG response was predominantly IgG1. CF and control subjects had similar IgG and IgG1 antibody levels. The PRP-specific IgG response was predominantly IgG2. In contrast to tetanus toxoid results, CF patients had lower geometric mean level of PRP-specific IgG compared to normal controls ($p = 0.0036$). ELISA results were confirmed by liquid-phase ³H-PRP-binding assay: CF patients had a geometric mean serum antibody level of 395 versus 922 ng/ml in controls ($p = 0.0044$). PRP-specific IgG2 levels were also depressed in CF patients ($p = 0.03$). CF patients had a lower prevalence of the A2m(2) allotype than the local racially matched control sample ($p < 0.025$). Other allotype prevalences including G2m(n) and Km(1) were similar. Impaired IgG2 antibody responses to microbial polysaccharide surface antigens in CF patients might predispose them to persistent endobronchial infection and lead to production of nonopsonizing isotype responses. The potential role of A2m(2), coded for in the H chain locus on chromosome 14, is unknown, but could be related to mucosal IgA2 antibody responses. (*Pediatr Res* 22: 708-713, 1987)

Abbreviations

CF, cystic fibrosis
ELISA, enzyme-linked immunosorbent assay
TT, tetanus toxoid
PRP, *Haemophilus influenzae* type b polyribophosphate
PA, *Pseudomonas aeruginosa*
LPS, lipopolysaccharide
Gm, Am, Km, immunoglobulin G and A heavy chain, and
κ light chain, allotypes

HSA, human serum albumin
PBS, phosphate-buffered saline
NGS, normal goat serum
OPD, *o*-phenylenediamine
EU, ELISA units
Mab, monoclonal antibody

Chronic airways infection with PA occurs in more than 80% of patients with CF and is responsible for most morbidity and mortality seen in this disease. The unique anatomic and bacteriologic features of this infection strongly suggest a local immune deficiency. The most promising candidate for such an immune deficiency is PA-specific nonopsonizing or blocking antibody detected in PA-infected CF patients (1, 2), since opsonic IgG antibodies specific for PA surface antigens such as LPS are essential for normal pulmonary and systemic phagocytic clearance (3, 4).

Several mechanisms might underlie the production of blocking antibodies to PA by CF patients. Proteolytic degradation of IgG into dysfunctional fragments has been demonstrated *in vivo* in CF bronchial lavage fluids and *in vitro* upon incubation with PA-derived elastase (5, 6). Changes in Fc-mediated effector function secondary to altered glycosylation of CF immunoglobulin could conceivably play a role (7). However, neither of these explanations account for the antigenic restriction of the opsonic defect to PA. In contrast, a change in the pattern (isotype distribution) of the antibody response subsequent to initial colonization might result in production of blocking antibody, an alteration capable of causing an antigen-specific immune deficiency (8-10).

In studying this phenomenon, we were struck by the unexpected finding that patients with CF who were not infected with PA appeared to have an impaired humoral immune response to PA LPS, as manifested by subnormal levels of PA LPS-specific IgG2 antibodies and an apparent redistribution of the response to nonopsonizing IgG4 (8).

The adult human antibody response to polysaccharide antigens is normally at least partly restricted to the IgG2 subclass (11-16). It seems possible that an inability to respond with an adequate IgG2 response could predispose to infection with polysaccharide-encapsulated pathogens such as PA. Herein, we explored the hypothesis that CF patients have a deficient response to polysaccharide antigens by measuring natural IgG and IgG1-4 subclass antibody levels to PRP, and a control protein antigen, TT, in older patients with CF compared to normal subjects. We also examined allotypic markers on CF immunoglobulin to assess possible genetic contributions to impaired responses.

Received March 23, 1987; accepted August 4, 1987.

Correspondence and reprints R. B. Moss, M.D., Children's Hospital-Stanford, 520 Sand Hill Road, Palo Alto, CA 94304.

Supported by grants from the Cystic Fibrosis Foundation and Cystic Fibrosis Research, Inc.

¹ Presented in part at the American Academy of Allergy & Immunology, Washington, D.C., February 23, 1987, and the Society for Pediatric Research, Anaheim, CA, April 29, 1987.

METHODS

Serum samples. Serum samples for antibody analysis were obtained from 24 older patients with CF (14 males, 10 females, median age 24, range 16–37) attending the Stanford CF Center clinic. All study subjects were >16 yr of age to control for ontogenetic variations in immune responses. Sixteen CF patients had chronic endobronchial PA infection, as defined by ≥ 3 positive sputum cultures of ≥ 1 yr duration plus an abnormally high humoral immune response to PA LPS (17). Eight CF patients not infected with PA were also studied; these patients' sputum cultures grew a variety of other pathogenic bacteria, including *Staphylococcus aureus*, nontypable *Haemophilus influenzae* or *Haemophilus parainfluenzae*, and various species of Enterobacteriaceae.

Haemophilus influenzae type b was not consistently cultured from the sputum of any of the study patients. One of the CF subjects' sputum culture grew this organism at the time of blood sampling, but approximately 25% had a history of intermittently positive cultures in the past. The control group consisted of 20 healthy adult hospital workers (nine males, 11 females, median age 30½ yr, range 25–50 yr) involved in the care of CF patients. None of the study subjects had received the *Haemophilus* type b PRP vaccine or a tetanus booster immunization within the preceding 10 yr.

Activation and coupling of PRP. Approximately 90 µg lyophilized PRP (HIB-Immune, Lederle, Pearl River, NY) were dissolved in 100 µl distilled water and alkalized by addition of 0.5 ml 0.01 N NaOH–0.001% phenolphthalein. The mixture was transferred to a glass tube containing 0.5 mg cyanuric chloride for activation. Then 0.1 ml 0.1% polylysine was added (18). Coupled antigen was diluted in normal saline to a final concentration of 1 µg/ml for solid phase adsorption.

ELISA for PRP-specific IgG. Assays were performed in 96-well flat-bottom polyvinylchloride microtiter plates (Dynatech, Alexandria, VA). Wells were coated with coupled PRP by incubation of 100 µl/well overnight at 4° C. Following aspiration, open well sites were blocked by incubation with 200 µl/well 0.1% HSA-PBS–0.01% thimerosal for 1 h at 37° C. Following aspiration and three washes with PBS–0.05% Tween–thimerosal, human test sera diluted 1/10 and 1/100 in 10% NGS–PBS–thimerosal diluent were added to wells in duplicate and incubated for 3 h at 4° C. After aspiration and washing, monospecific affinity-purified goat antihuman IgG-peroxidase conjugate (Sigma, St. Louis, MO) 1/1000 in 10% NGS was added and incubated for 1 h at 37° C. Following the final aspiration and washing, enzyme substrate OPD was added and incubated for 10–20 min at 22° C. The reaction was stopped with sulfuric acid and the resultant absorbance recorded at 490 nm using an automated microplate spectrophotometer.

The assay was standardized with a reference antiserum containing high-titer *H. influenzae* type b antibodies quantified for all precipitating isotypes (S. Klein, US Standard human anti-*H. influenzae* type b capsular polysaccharide antibody, lot 1983, Office of Biologics, FDA, Bethesda, MD) (19). Net absorbances were derived by subtracting the higher of background readings obtained either by incubation of serum in paired wells without antigen or antigen-coated wells incubated with filler instead of serum. Background readings obtained from PRP-polylysine exposed to secondary antibodies were in the 0.05–0.1 OD₄₉₂ nm range. Results, expressed in arbitrary EU, were determined by interpolation of test sera net absorbance values to the standard curve.

ELISA for PRP specific-IgG1-4. The IgG ELISA was modified by the use of murine Mabs specific for individual human IgG 1–4 subclasses. Results for each subclass antibody assay were rendered comparable by adjusting dilutions of each antiisotypic Mab employed to yield identical net absorbance in binding to solid-phase adsorbed homologous purified human IgG subclass myelomas, as described elsewhere in detail (20). This assay

provides semiquantitative estimates of the relative quantity and distribution of various IgG subclass antibodies for a given antigen, provided that these antibodies have similar affinities for that antigen.

Equivalent signal output (net absorbance) for human IgG1-4 subclass-specific Mabs was established just prior to testing of serum samples for PRP IgG1-4 antibody levels. The equivalent dilutions and Mabs employed were as follows: 1/5000 for Mab 912 anti-IgG1 (Hybritech, La Jolla, CA); 1/3000 for Mab GOM-1 anti-IgG2 (Seward, Birmingham, England); 1/20,000 for Mab 211 anti-IgG3 (Bethesda Research Laboratories, Gaithersburg, MD); and 1/16,000 for Mab SK-44 anti-IgG4 (Bio-Yeda, Rehovot, Israel). Antibodies were diluted in 10% NGS. Mabs binding to homologous myelomas demonstrated parallel titration slopes with that of the reference serum binding to PRP.

Following serial incubation of PRP, HSA, and test sera diluted ½ and ¼ for 5 h at 4° C, Mabs were added and incubated overnight at 4° C. After aspiration and washing, affinity-purified goat antimouse IgG-peroxidase conjugate (Boehringer Mannheim, Indianapolis, IN) 1/1000 in 10% NGS was added and incubated 1 h at 37° C. The reaction was then developed with OPD as described above. Results were standardized with the reference serum and expressed in arbitrary EU.

As with the IgG ELISA, negative controls included wells exposed to filler and serum, or antigen and filler. Nonspecific binding was generally in the 0.1–0.2 OD₄₉₂ nm range. In addition, unfractionated human serum containing no detectable antibody to PRP (<15 ng/ml by radioactive antigen-binding assay; total IgG level 550 mg/dl) was also studied at the same dilutions and found not to result in higher nonspecific binding levels than the other negative controls. The apparent insensitivity of the PRP IgG2 subclass ELISA resulted from the employment of the secondary antibody GOM-1 at its antiisotypic "equivalent" dilution of 1/3000 (*vide supra*) (20). This was demonstrated in experiments showing PRP antibody-positive serum titration curves yielding net absorbances well over three times negative controls at dilutions of $\geq 1/32$ if GOM-1 was employed at dilutions of 1/500 or 1/1000 (data not shown). Similar net absorbance specific:nonspecific binding ratios were generated at the final ¼ serum screening and 1/3000 GOM-1 dilutions employed.

Radioactive antigen-binding assay. Total precipitating antibody to PRP was measured by a radioactive antigen binding assay using tritium-labeled PRP as described (21). Freshly prepared ³H-PRP (310 nCi/180 ng/ml) was generously provided by Dr. Porter Anderson, University of Rochester Medical Center, Rochester, NY.

ELISA for TT-specific IgG and IgG1-4. The TT IgG assay was performed as described above for PRP, with the following exceptions: 1) TT (Connaught, Swiftwater, PA) was directly adsorbed to wells by incubation for 1 h at 37° C. The TT antigen contained 8 I.U./ml and 26.5 µg/ml protein by the Folin phenol method. For adsorption, a protein concentration of 1 µg/ml was used. 2) Test sera were diluted 1/200 and 1/1000. 3) The assay was standardized by a reference curve of a human tetanus immune globulin preparation (Hyper-Tet, Cutter, Berkeley, CA) containing 250 antitoxin U/ml. Results are given in arbitrary EU derived from interpolation of test sera to the standard curve.

The TT IgG1-4 subclass assay was performed as described for PRP, except that serum dilutions of 1/50 and 1/200 were used. As for the TT IgG assay, results were standardized using Hyper-Tet and are also given in arbitrary EU. Isotype and antibody dilution curves were parallel, as were IgG1 (the major isotype) and IgG antibody curves.

Immunoglobulin allotypes. These were determined by hemagglutination inhibition in the laboratory of G. de Lange. The following allotypes were identified: G1m (z, a, x, f), G2m (n), G3m (g1, g5, b1, b5, s, t, c3, c5), A2m (1, 2), and Km (1, 3). In certain sera, further markers (b0, b3, b4) were tested. Sera were screened for anti-Gm and anti-Km antibodies.

Statistical analysis. Geometric mean antibody levels were compared by the Mann-Whitney U test after logarithmic transformation, using two-tailed *p* values for significance. Results were also analyzed using two-tailed Student's *t* test on log transformed antibody values, but the nonparametric test results are reported due to non-Gaussian distribution histograms of some of the data. Parametric and nonparametric significance values were in close agreement and in no case were discordant conclusions reached by the two methods. Allotype prevalences were compared by the χ^2 statistic with Yates' correction of continuity. Statistical calculations were performed using Statview 512 statistical software package (BrainPower, Inc., Calabasas, CA).

RESULTS

Patients with CF had similar TT IgG antibody levels to controls (Fig. 1A). The geometric mean value for the CF group was 8075 versus 6883 EU in the control group ($p > 0.2$). Sixteen CF patients who were chronically infected with PA had similar TT antibody levels to eight CF patients without PA infection (geometric mean = 8390 versus 6353 EU, $p = 0.13$).

The IgG subclass antibody response to TT in healthy adults was predominantly IgG1, with minor contributions from IgG2-4. The hyperimmune standard globulin preparation used to standardize the ELISA contained the following distribution of IgG subclass TT antibodies, as determined in three separate experiments: 52–58% IgG1, 15–17% IgG2, 12–14% IgG3, and 15–17% IgG4. CF patients demonstrated a similar distribution of humoral response to TT, with IgG1 predominance. TT IgG and IgG1 antibody levels showed a high degree of correlation for all subjects ($r = +0.87$, $p < 0.001$).

Individual serum IgG1 antibody levels to TT are shown in Figure 1B. The geometric mean TT IgG1 antibody levels were similar in the patients with CF compared to normal controls (327 versus 398 EU, respectively). CF patients infected with PA and CF patients without PA infection had similar levels of TT IgG1 (402 versus 218 EU, $p = 0.14$).

The PRP-specific IgG antibody levels determined by ELISA are shown in Figure 2A. Unlike the results seen with TT, CF patients as a group had significantly lower serum IgG antibody

Fig. 1. A, serum TT-specific IgG antibody levels in CF patients (circles) and controls (triangles); CF patients chronically infected with PA are indicated by closed circles. Geometric mean antibody levels were similar in the two groups. B, TT-specific IgG1 antibody levels in CF patients and controls. Geometric means were similar in the two groups. NI, controls.

Fig. 2. A, serum PRP-specific IgG antibody levels in CF patients (circles) and controls (triangles); CF patients chronically infected with PA are indicated by closed circles. The geometric mean was lower in CF patients ($p = 0.0036$). B, serum PRP-specific IgG2 antibody levels in CF patients and controls. The geometric mean was lower in CF patients ($p = 0.03$).

levels to PRP compared to normal subjects. This occurred despite the fact that one control subject had a PRP IgG level below the detection limit of the standard curve (30 ng/ml). The geometric mean IgG PRP antibody level in patients with CF was 1140 versus 2310 EU in controls ($p = 0.0036$). CF patients infected with PA and CF patients without PA infection had similar PRP IgG antibody levels (geometric mean = 1110 versus 1200).

To further examine the response to PRP, a radioantigen-binding assay detecting antibodies of all isotypes binding to ^3H -PRP and precipitable by 12.5% polyethylene glycol was used (21). In this assay, CF patients also demonstrated a significantly lower response (Table 1). The geometric mean antibody level in CF patient group was 395 versus 922 ng/ml in the control group ($p = 0.0044$). The results of the ^3H -PRP radioantigen-binding assay and the PRP IgG ELISA correlated well ($r = +0.65$, $p < 0.001$). The control subject who had the lowest level of PRP-specific IgG by ELISA (see above) had 88 ng/ml ^3H -PRP binding antibody in the radioantigen assay. Six patients with CF (25%) and two controls (10%) had ^3H -PRP binding levels <150 ng/ml.

The normal adult IgG antibody response to PRP was predominantly IgG2, although a variable minority contribution from IgG1 was apparent in some individuals in IgG1-4 subclass ELISA using equivalent signal-generating dilutions of subclass-specific secondary monoclonal antibodies (Fig. 3). The distribution of IgG subclasses in the reference serum was determined in three experiments to be approximately 28–31% IgG1, 51–52% IgG2, 10–13% IgG3, and 7–8% IgG4. The distribution of CF patient IgG subclass antibody response to PRP appeared similar to healthy controls. In particular, there was no clear-cut evidence of compensatory shifts in IgG subclass antibody response toward

IgG1, 3, or 4 (Fig. 3). PRP IgG and IgG2 antibody levels for all subjects were significantly correlated ($r = +0.74$, $p < 0.001$).

As with PRP IgG antibodies in ELISA and ^3H -PRP binding precipitating antibodies, PRP IgG2 antibody levels determined by subclass ELISA were lower in CF patients than in controls (Fig. 2B). Two patients with CF had PRP IgG2 antibody levels below the detection limit of the assay; these two patients' sera had ^3H -PRP binding levels of 144 and 400 ng/ml and PRP IgG levels of 400 and 330 ng/ml, respectively. The geometric mean PRP IgG2 antibody level in the CF group was 2727 versus 7526 EU in the control group ($p = 0.029$). CF patients infected with PA and those without PA infection had similar levels of PRP IgG2 antibodies (geometric mean = 2425 versus 3450 EU, $p = 0.3$).

Immunoglobulin allotype determinations were done on serum samples from the 44 subjects assayed for antibody responses plus an additional 122 CF and 76 controls. All subjects were more than 6 months of age. Control group sera were obtained from subjects with other respiratory diseases (mostly asthma) followed in the Stanford Pediatric Pulmonary clinic. Hispanic, black, and Oriental subjects were excluded from allotype data analysis.

Frequencies of all Gm, Km, and A2m(1) allotypes were not significantly different in the two groups. There was a possible trend toward lower frequency of Km(1) in the CF sample group (14.3 versus 21.1%, $p > 0.1$). One CF and one control subject were excluded from A2m analysis due to IgA deficiency. A2m(2) was present in 4.1% of the CF group versus 13.8% of the control group (χ^2 with continuity correction = 6.138, $p < 0.025$).

Anti-Km(1) antibody was present in 1 CF subject who was

Km(3+). Anti-G1m(a) antibodies were present in 1 CF and 2 control subjects who were all G1m(f+). Finally, anti-G3m(b0,b1) was present in one CF subject who was G3m(g1, g5+).

DISCUSSION

The purpose of this study was to examine the natural antibody response of CF subjects to polysaccharide and protein antigens in light of our previous finding of subnormal PA LPS IgG2 antibody proportions and levels in CF patients not infected with PA (8). In a subsequent study, we noted a high correlation between PA LPS IgG2 antibody levels and total serum IgG2 protein levels. CF patients without PA infection tended toward low serum IgG2 levels, with two in 10 being IgG2 deficient (22). One interpretation of these findings is that CF patients may have an impaired ability to mount a normal IgG2-restricted antibody response to polysaccharide antigens. To study this question, we measured serum antibody levels to two ubiquitous antigens, the PRP capsular polysaccharide of *H. influenzae* type b, and the protein antigen, TT.

The systemic humoral immune response to various bacterial, viral, dietary, and drug protein antigens, including TT and influenza vaccines, has been extensively studied in patients with CF (23). These studies have uniformly suggested normal responses in CF patients. Our ELISA results for TT-specific IgG confirm that adult CF patients have antibody levels to this antigen, due to universal childhood immunization, which are similar to controls.

The normal IgG antibody response to TT is predominantly

Table 1. Antibody responses to TT and PRP in patients with CF and controls

Antibody (unit)	Group				
	CF (n = 24)	Mean	Normal (n = 20)	Mean	p
	GM*	log ₁₀ ± SD	GM	log ₁₀ ± SD	(2-tailed)
TT IgG (EU)	8075	3.91 ± 0.33	6883	3.84 ± 0.45	NS
TT IgG1 (EU)	327	2.52 ± 0.43	398	2.60 ± 0.50	NS
^3H -PRP precipitating (ng/ml)	395	2.60 ± 0.41	922	2.97 ± 0.45	0.0044
PRP IgG (EU)	1143	3.06 ± 0.29	2313	3.36 ± 0.60	0.0036
PRP IgG2 (EU)	2727	3.44 ± 0.65	7526	3.88 ± 0.55	0.03

* Geometric mean titer.

Fig. 3. PRP-specific IgG1-4 serum antibody titration curves of CF patients (dashed lines) and controls (solid lines). Net absorbance (OD490) have been equilibrated by the myeloma method to reflect approximate distribution of serum antibodies (see text). Low PRP-specific IgG2 levels in CF patients are not compensated by increases in other isotype antibodies.

IgG1 (24–27). Our data confirm these studies and indicate that patients with CF also respond to TT with a dominant IgG1 response, which is quantitatively similar to that of controls. A variable minority contribution of IgG2–4 TT antibodies may be seen in individual subjects, with IgG4 being particularly important in some (25–27).

Examination of the antibody response to polysaccharide antigens highlights a number of qualitative and quantitative differences vis-à-vis the response to proteins. First, the isotype pattern of response is qualitatively distinct, with predominance of IgG2 antibodies (11–16). Second, the ontogeny of response differs, with a later maturation of immune responsiveness, which for some antigens may extend well into the latter years of childhood (28, 29). To eliminate the ontogenetic factor, we restricted our investigation in this study to immunologically adult (>16 yr old) subjects.

Antibodies to *H. influenzae* type b capsular PRP are present in virtually all unimmunized adults at levels sufficient to prevent invasive *H. influenzae* type b disease (≥ 150 ng/ml precipitable antibody) (19). In contrast to immunization-induced TT antibodies, PRP antibodies are thought to be induced as a result of natural exposure to enteric microbial flora, especially *Escherichia coli*, which has a capsular polysaccharide antigen (K100) which is cross-reactive with PRP (30).

The major finding of this study is that adult CF patients have significantly lower levels of serum PRP IgG antibodies than controls (Fig. 2). The normal human IgG antibody response to PRP is partly restricted to IgG2, with a variable minority contribution from IgG1 (12, 31). Recently, Weinberg *et al.* (32) demonstrated that IgG2 subclass PRP antibodies were functional in *in vitro* bactericidal and *in vivo* rat bacteremia protection assays. Similar data supporting a protective biologic role for polyclonal or monoclonal human IgG2 antibodies to other polysaccharides, such as type III group B *Streptococcus* capsule and *Pseudomonas aeruginosa* LPS, have been obtained (Masuho Y, personal communication) (33). Moreover, a number of recent clinical studies suggest that deficiency of IgG2 (or pathogen-specific IgG2 antibodies even when IgG2 levels are normal) may predispose to sinopulmonary infection (34, 35). CF patients in our study, as with the normal controls, made a predominantly IgG2 response, without evidence of a compensatory shift to IgG1 or other IgG isotypes. As with IgG antibodies, PRP-specific IgG2 levels were generally lower in CF patients. These findings, particularly in light of our previous observations regarding *P. aeruginosa* LPS antibodies (8), suggest IgG2 responses to polysaccharide antigens, of potential functional importance, might be impaired in CF patients.

The human response to PRP includes IgM and IgA antibodies, which we did not directly measure. However, we confirmed the lower PRP IgG antibody levels in CF patients determined by ELISA by radioantigen-binding assay (21), suggesting that compensatory or increased systemic IgA or IgM antibody responses do not occur to a major degree in these patients.

An alternative explanation for lower PRP antibody levels in CF patients is that antigenic exposure might somehow be less than that experienced by normal individuals. In particular, since natural PRP antibodies are thought to arise from responses to normal gut flora (especially *E. coli*) (30), could frequent antibiotic treatment deprive the CF individual of this antigenic stimulation? Available data on colonization rates and immune responses to enteric bacteria, including *E. coli*, in patients with CF would appear to preclude this possibility, since these are both normal or increased in CF patients despite vigorous antibiotic treatment (36–39). Increased direct PRP antigenic stimulation in CF patients seems unlikely, however, given the fact that most *H. influenzae* respiratory tract colonization and infection in CF patients is due to nontypeable, acapsulate strains (40).

To investigate a possible genetic basis for this impairment, we examined allotypes of CF and control immunoglobulins. We found that the allotypic light chain marker Km(1), previously

associated in certain populations with enhanced immune responsiveness to PRP and other polysaccharide antigens (41–45), had a lower prevalence in our CF sample population than controls. The exclusion of Hispanic individuals, however, eliminated the difference, rendering conclusions difficult. It thus seems possible that lower IgG2 responses may have a genetic component detectable by Km(1) allotype analysis. We did not find any differences in the prevalence of the IgG2 heavy chain allotype G2m(n), which Ambrosino *et al.* (45), but not Granoff *et al.* (43, 44), have correlated with PRP immune responsiveness.

Another allotype, found on IgA2 heavy chains, A2m(2) (46), was also less frequent in CF patients. A2m(2) is relatively rare in Caucasian populations and more common in blacks and Orientals (47). We excluded the latter racial groups from our analysis, and the presence or absence of Hispanic individuals did not alter the results. To our knowledge, no studies of IgA allotypes and immune responses have been published. It is intriguing to note, however, that IgA2 plays a prominent role in local mucosal IgA production (48). In particular, IgA2 antibodies appear to provide the major portion of the mucosal IgA antibody response to bacterial LPS and staphylococcal ribitol teichoic acid (15, 49). Aberrations in the secretory IgA system have been reported in CF patients, but no data on IgA subclass composition were presented (50). Pathology in IgA, IgG, or both isotypes could play a role in development of CF lung disease. Independent, complementary functions for secretory IgA and IgG subclass antibodies in pulmonary defense are currently postulated via prevention of mucosal microbial adherence and colonization by the former, and subsequent opsonophagocytosis if necessary by the latter (51).

In future studies it will be necessary to examine primary and anamnestic responses of CF patients to polysaccharide vaccines. It will also be useful to study fluctuations in bacterial polysaccharide-specific antibody levels over time in relation to respiratory tract colonization and episodes of infection. At present, the role of a rather restricted but potentially important immune response, impaired IgG2 antibody responses to polysaccharide antigens, in predisposing CF patients to chronic airway infection remains speculative. Continued study of host responses seems likely to provide additional clues to those lines of inquiry focused solely on bacterial virulence factors in understanding the uniquely circumscribed nature of pulmonary infection in CF.

REFERENCES

1. Thomassen MJ, Boxerbaum B, Demko CA, Kuchenbrod PJ, Dearborn DG, Wood RE 1979 Inhibitory effect of cystic fibrosis serum on *Pseudomonas* phagocytosis by rabbit and human alveolar macrophages. *Pediatr Res* 13:1085–1088
2. Fick RB, Naegel GP, Matthay RA, Reynolds HY 1981 Cystic fibrosis *Pseudomonas* opsonins. Inhibitory nature in an *in vitro* phagocytic assay. *J Clin Invest* 68:899–914
3. Young LS, Armstrong D 1972 Human immunity to *Pseudomonas aeruginosa*. I. *In vitro* interaction of bacteria, polymorphonuclear leukocytes, and serum factors. *J Infect Dis* 126:257–276
4. Reynolds HY, Kazmierowski JA, Newball HH 1975 Specificity of opsonic antibodies to enhance phagocytosis of *Pseudomonas aeruginosa* by alveolar macrophages. *J Clin Invest* 56:376–385
5. Fick RB, Naegel GP, Squier SU, Wood RE, Gee JBL, Reynolds HY 1984 Proteins of the cystic fibrosis respiratory tract. Fragmented immunoglobulin G opsonic antibody causing defective opsonophagocytosis. *J Clin Invest* 74:236–248
6. Fick RB, Baltimore RS, Squier SU, Reynolds HY 1985 IgG proteolytic activity of *Pseudomonas aeruginosa* in cystic fibrosis. *J Infect Dis* 51:589–598
7. Margolies R, Boat TF 1983 The carbohydrate content of IgG from patients with cystic fibrosis. *Pediatr Res* 17:931–935
8. Moss RB, Hsu YP, Sullivan MM, Lewiston NJ 1986 Altered antibody isotype in cystic fibrosis: possible role in opsonic deficiency. *Pediatr Res* 20:453–459
9. Fick RB, Olchowski J, Squier SU, Merrill WW, Reynolds HY 1986 Immunoglobulin G subclasses in cystic fibrosis. *Am Rev Respir Dis* 133:418–422
10. Shryock TR, Molle JS, Klinger JD, Thomassen MJ 1986 Associations with phagocytic inhibition of anti-*Pseudomonas aeruginosa* immunoglobulin G antibody subclass levels in serum from patients with cystic fibrosis. *J Clin Microbiol* 23:513–516
11. Yount WJ, Dörner MM, Kunkel HG, Kabat EA 1968 Studies on human antibodies. IV. Selective variations in subgroup composition and genetic markers. *J Exp Med* 127:633–646

12. Johnston RB, Anderson P, Rosen ES, Smith DH 1973 Characterization of human antibody to polyribosephosphate, the capsular antigen of *Haemophilus influenzae* type B. Clin Immunol Immunopathol 1:234-240
13. Riesen WF, Skvaril F, Braun DG 1976 Natural infection of man with group A streptococci. Scand J Immunol 5:383-390
14. Siber GR, Schur PH, Aisenberg AC, Weitzman SA, Schiffman G 1980 Correlation between serum IgG2 concentrations and the antibody response to bacterial polysaccharide antigens. N Engl J Med 303:178-182
15. Hammarstrom L, Persson MAA, Smith CIE 1985 Immunoglobulin subclass distribution of human anti-carbohydrate antibodies: aberrant pattern of IgA deficient donors. Immunology 54:821-826
16. Barrett DJ, Ayoub EM 1986 IgG2 subclass restriction of antibody to pneumococcal polysaccharides. Clin Exp Immunol 63:127-134
17. Moss RB, Hsu YP, Lewiston NJ, Curd JG, Milgrom H, Hart S, Dyer B, Larrick JR 1986 Association of systemic immune complexes, complement activation, and antibodies to *Pseudomonas aeruginosa* lipopolysaccharide and exotoxin A with mortality in cystic fibrosis. Am Rev Respir Dis 133:648-652
18. Gray BM 1979 ELISA methodology for polysaccharide antigens: protein coupling of polysaccharides for adsorption to plastic tubes. J Immunol Methods 28:187-192
19. Robbins JB, Parke JC, Schneerson R, Whisnant JK 1973 Quantitative measurement of "natural" and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. Pediatr Res 7:103-110
20. Moss RB, Hsu YP, Kwasnicki JM, Sullivan MM, Reid MJ 1987 Isotypic and antigenic restriction of the blocking antibody response to rye grass pollen: correlation of rye group I antigen-specific IgG1 with clinical response. J Allergy Clin Immunol 79:387-398
21. Kuo JS-C, Mouli N, Schwabbe RS, McCoy DW 1981 A radioactive antigen-binding assay for the measurement of antibody to *Haemophilus influenzae* type b capsular polysaccharide. J Immunol Methods 43:35-47
22. Moss RB 1987 Hypergamma globulinemia in cystic fibrosis. Role of *Pseudomonas* endobronchial infection. Chest 91:522-26
23. Moss RB 1983 Immunology of cystic fibrosis. In: Lloyd-Stoll JD (ed) Textbook of Cystic Fibrosis. Wright/PSG, Boston, pp 53-115
24. Stevens R, Dichek D, Keld B, Heiner DC 1983 IgG1 is the predominant subclass of *in vivo* and *in vitro*-produced antitetanus toxoid antibodies and also serves as the membrane IgG molecules for delivering inhibitory signals to antitetanus toxoid-producing B cells. J Clin Immunol 3:65-69
25. Seppala HJT, Routonen N, Sarnesto A, Mattila PA, Makela O 1984 The percentage of six immunoglobulin isotypes in human antibodies to tetanus toxoid: standardization of isotype specific second antibodies in solid-phase assay. Eur J Immunol 14:868-875
26. Rubin RL, Tang FL, Lucas AH, Spiegelberg HL, Tan EM 1986 IgG subclasses of antitetanus antibodies in adult and newborn normal subjects and in patients with systemic lupus erythematosus, Sjogren's syndrome, and drug-induced autoimmunity. J Immunol 137:2522-2527
27. Dengrove J, Lee EJ, Heiner DC, St Geme JW, Leake R, Baraff LJ, Ward JI 1986 IgG and IgG subclass specific antibody response to diphtheria and tetanus toxoids in newborns and infants given DTP immunization. Pediatr Res 20:735-739
28. Freijd A, Hammarstrom L, Persson MAA, Smith CIE 1984 Plasma antipneumococcal antibody activity of the IgG class and subclass in otitis prone children. Clin Exp Immunol 56:233-238
29. Hammarstrom L, Granstrom M, Molloy R, Oxelius V, Persson MA, Smith CIE 1985 Ontogeny of IgG2 antibodies against *S. aureus* teichoic acid in normal and immunodeficient children. Acta Paediatr Scand 74:126-130
30. Robbins JB, Schneerson R, Glode MP, Vann W, Schiffer MS, Liu T-Y, Parke JC, Hundley C 1975 Cross-reactive antigens and immunity to diseases caused by encapsulated bacteria. J Allergy Clin Immunol 56:141-151
31. Shackelford PG, Granoff DM, Nelson SJ, Scott MG, Smith DS, Nahm MH 1987 Subclass distribution of human antibodies to *Haemophilus influenzae* type b capsular polysaccharide. J Immunol 138:587-592
32. Weinberg GA, Granoff DM, Nahm MH, Shackelford PG 1986 Functional activity of different IgG subclass antibodies against type b capsular polysaccharide of *Haemophilus influenzae*. J Immunol 136:4232-4236
33. Givner LB, Baker CJ, Edwards MS 1987 Type III Group B *Streptococcus* functional interaction with IgG subclass antibodies. J Infect Dis 155:532-539
34. Berger M 1987 Immunoglobulin G subclass determination in diagnosis and management of antibody deficiency syndromes. J Pediatr 110:325-328
35. Lane PJ, MacLennan CM 1986 Impaired IgG2 antipneumococcal antibody responses in patients with recurrent infection and normal IgG2 levels but no IgA. Clin Exp Immunol 65:427-433
36. Seidman EJ, Mossovich LL, Neter E 1975 Colonization of *Enterobacteriaceae* of the respiratory tract of children with cystic fibrosis of the pancreas and their antibody response. J Pediatr 87:528-533
37. Macrone AB, Pier GB, Pennington JE, Matthews WJ, Goldmann DA 1981 Mucoid *Escherichia coli* in cystic fibrosis. N Engl J Med 304:1445-1449
38. Roy CC, Delage G, Fontaine A, Robitaille L, Chartrand L, Weber A, Morin CT 1979 The fecal microflora and bile acids in children with cystic fibrosis. Am J Clin Nutr 32:2404-2409
39. Holby N, Hertz JB 1979 Precipitating antibodies against *Escherichia coli*, *Bacteroides fragilis* ss, *Thetainomeron*, and *Pseudomonas aeruginosa* in serum from normal persons and cystic fibrosis patients. Acta Paediatr Scand 68:495-500
40. Holby N, Killian M 1976 *Haemophilus* from the lower respiratory tract of patients with cystic fibrosis. Scand J Respir Dis 57:103-107
41. Pandey JP, Virella G, Loadholt G, Fudenberg HH, Kyong CU, Galbraith RM 1979 Association between Ig allotypes and immune responses to *Haemophilus influenzae* and meningococcal polysaccharides. Lancet i:190-194
42. Pandey JP, Zollinger WD, Fudenberg HH, Loadholt CB 1981 Immunoglobulin allotypes and immune response to meningococcal group B polysaccharide. J Clin Invest 68:1378-1380
43. Granoff DM, Pandey JP, Boies E, Squires J, Munson RS, Suarez B 1984 Response to immunization with *Haemophilus influenzae* type b polysaccharide-pertussis vaccine and risk of *Haemophilus meningitis* in children with the Km(1) immunoglobulin allotype. J Clin Invest 74:1708-1714
44. Granoff DM, Shackelford PG, Pandey JP, Boies EG 1986 Antibody responses to *Haemophilus influenzae* type b polysaccharide vaccine in relation to Km(1) and G2m(23) immunoglobulin allotypes. J Infect Dis 154:257-264
45. Ambrosino DM, Schiffman G, Gotschlich EC, Schur PH, Rosenberg GA, Delange G, van Loghem E, Silber GR 1985 Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. J Clin Invest 75:1935-1942
46. Wang AC, van Loghem E, Shuster J 1973 Serological and biochemical investigations of a new genetic marker of human immunoglobulins determined by an allele at the alpha-2 locus. Fed Proc 32:1003(abstr)
47. Van Loghem E, Biewenga J 1983 Allotypic and isotypic aspects of human immunoglobulin A. Mol Immunol 20:1001-1007
48. Delacroix DL, Dive C, Rambaud JC, Vaerman JP 1982 IgA subclasses in various secretions and in serum. Immunology 47:383-385
49. Brown TA, Mestecky J 1985 Immunoglobulin A subclass distribution of naturally occurring salivary antibodies to microbial antigens. Infect Immun 49:459-462
50. Wallwork JC, McFarlane H 1976 The SIgA system and hypersensitivity in patients with cystic fibrosis. Clin Allergy 6:349-358
51. Fick RB, Hunninghake GW 1986 Antimicrobial defense of the lung: importance of secretory immunoglobulin A and opsonic immunoglobulin G antibodies. In: Simmons DH (ed) Current Pulmonology 7. Year Book Medical Publishers, Inc., Chicago, pp 165-198