Serotype-Specific Serum IgG Antibodies to Lipopolysaccharides of *Pseudomonas aeruginosa* in Cystic Fibrosis: Correlation to Disease, Subclass Distribution, and Experimental Protective Capacity

URS B. SCHAAD, ALOIS B. LANG, JOANNA WEDGWOOD, UELI BUEHLAMNN, AND
EMIL FUERER

Department of Pediatrics (Inselspital), University of Berne [U.B.S., J.W., U.B.], and Swiss Serum and Vaccine
Institute, Berne [A.B.L., E.F.], Switzerland

ABSTRACT. Various studies have demonstrated pronounced systemic IgG response to *Pseudomonas aeruginosa* (PA) infection in cystic fibrosis (CF). However, antibody response to serotype-specific lipopolysaccharides (LPS) has never been studied. ELISA for detection of IgG antibodies to LPS of nine PA-serotypes and to toxin A were performed with serum of 78 CF patients. Anti-LPS profiles of antibodies were confirmed by SDS-PAGE and immunoblotting techniques. The most frequent PA-serotypes found were immunotypes (IT) IT-1 and IT-2, and Habs-3 and Habs-4. Ten patients without PA colonization showed no detectable antibody titers. In patients with chronic PA colonization ($n = 46$), these antibody titers were significantly ($p < 0.005$) higher than in patients with intermittent PA colonization ($n = 22$). Mean serum antibody titers to LPS of PA IT-1, IT-2, Habs-3, and Habs-4 correlated with duration of PA colonization and with disease severity. Subclass analysis of anti-LPS antibodies revealed elevated levels for all four IgG subclasses and for IgA₁. The IgG antibodies to LPS of PA proved to be protective in a murine burn wound sepsis model. We conclude that anti-LPS antibodies to specific PA serotypes in serum may be a sensitive measure of severity and prognosis of CF. Patients with CF show adequate functional immune response to LPS of PA, and it is possible that vaccination against PA before colonization could induce protective immunity. (*Pediatr Res* 27: 508–513, 1990)

Abbreviations

CF, cystic fibrosis
IT, immunotype
LPS, lipopolysaccharide
PA, *Pseudomonas aeruginosa*
Tox A, toxin A

PA is a persistent sputum pathogen in most patients with advanced CF (1). *Pseudomonas* pulmonary infections are the major determinant of quality and duration of life in these patients. Mechanisms of virulence and pathogenesis of PA are

extremely complex and include invasiveness and toxigenicity (2, 3). Moreover, direct and indirect evidence of induction of immunologic damage by PA has been repeatedly reported (2–5).

CF patients infected and colonized by PA produce substantial IgG serum antibody titers against a variety of PA antigens, including exoproteins (6, 7), cell surface structures (8, 9), outer-membrane proteins (10), water-soluble antigens (11, 12), and LPS (13, 14). The majority of these antibodies against PA antigens was shown to correlate with CF disease severity and prognosis (5–14). These antibodies are unable to stop the spread of bacteria in the lungs and the resultant tissue destruction in advanced CF.

The purpose of our investigations was to measure antibody response to serotype-specific LPS in patients with CF and to correlate these titers with PA colonization/infection and disease stage. Moreover, we compared IgG and IgA subclass distribution of antibodies against PA from sera of CF patients and healthy individuals immunized with PA conjugate vaccine. Finally, protection against PA infection in a murine burn wound sepsis model was studied by passive transfer of IgG derived from sera of CF patients and healthy individuals immunized with PA conjugate vaccine.

MATERIALS AND METHODS

Study patients. Between January 1987 and June 1988, 78 patients with CF attending the CF clinic at our hospital were studied. There were no inclusion or exclusion criteria regarding age or disease severity, but patients were not enrolled during acute bronchopulmonary exacerbation. The patients were 1–27 y old (mean $\pm$ SD, 14.7 ± 6.2 y), 40 were female, and 38 were male. Ten patients had no PA colonization, 22 patients were classified as intermittently colonized with PA on the basis of intermittent growth of PA in sputum, and 46 patients were classified as chronically colonized on the basis of three or more positive sputum cultures over at least 1 y. Each patient was only studied once. The trial was approved by the hospital ethics committee, and the patients or their parents gave informed consent.

Serum samples were obtained by venipuncture and frozen at -20°C until tested. The patients were assigned to various categories for each of the following criteria: age, duration of PA colonization, degree of underweight, CF score, radiographic score, and vital capacity. Correlations between the categories and mean serum antibody titers were assessed graphically. Underweight was determined as percent of predicted wt for age (15). The CF scoring system used included physical activity, findings

Received September 19, 1989; accepted January 3, 1990.

Correspondence and requests for reprints: Urs B. Schaad, M.D., Division of Pediatric Infectious Diseases, Department of Pediatrics, University of Berne, Inselspital, CH-3010 Berne, Switzerland.

Fig. 1. Distribution of serum antibody titers against LPS of IT-1 and Tox A in the three CF patient groups according to their PA colonization status: none ($n = 10$), intermittent ($n = 22$) and chronic ($n = 46$).

Table 1. Serum antibody titers to LPS of PA serotypes IT-1, IT-2, Habs-3, Habs-4, and Tox A in CF patients intermittently or chronically colonized with PA*

Antibody titer to:	Intermittent PA colonization ($n = 22$)	Chronic PA colonization ($n = 46$)	p value†
IT-1	1508 $\pm$ 1611 (0–5125)	2713 $\pm$ 1632 (60–5125)	<0.005
IT-2	374 $\pm$ 619 (0–2560)	1369 $\pm$ 1546 (0–5125)	<0.005
Habs-3	414 $\pm$ 485 (0–1280)	1158 $\pm$ 1191 (0–5120)	<0.005
Habs-4	463 $\pm$ 654 (0–2560)	1369 $\pm$ 1487 (0–5125)	<0.05
Tox A	247 $\pm$ 244 (0–844)	484 $\pm$ 457 (60–2200)	<0.005

* Values are mean $\pm$ SD (range).

† Wilcoxon-Mann-Whitney rank sum test.

on physical examination and in sputum, signs of pulmonary disease, radiologic lung involvement, and gastrointestinal manifestations (15, 16). Each of these variables was rated on a scale of 1 to 5 points, with the highest score indicating the best status. Posteroanterior and lateral chest radiographs were graded by a pediatric radiologist according to the Chrispin-Norman scoring system (17), ranging from 0 (normal chest roentgenogram) to 38 (maximal lung and chest abnormalities in CF). Vital capacity was measured in a volume-constant whole-body plethysmograph (18) and expressed as percentage of predicted value for ht.

Vaccine studies were performed in 18 healthy volunteers of both sexes 19–45 y old. Their written informed consent was obtained according to the approval of the institutional research committee.

Purification of antigens. Tox A was purified from PA-103 as

previously described (19). LPS was extracted from cell wall fragments of the nine most frequently isolated PA-serotypes (IT-1 to IT-7 according to Fisher, and Habs-3 and Habs-4) according to methods described in detail elsewhere (20). PA strains were serotyped by slide agglutination with suitably diluted antisera against the 17 O-type strains of the International Antigenic Typing System (21).

ELISA. ELISA for the quantitation of antibodies to LPS and Tox A in patients' serum samples were performed in the following manner (19). Microtiter plates (Dynatech, Kloten, Switzerland) were coated with 200 μ L of LPS (10 μ g/mL) of Tox A (10 μ g/mL) by incubation at 37°C for 3 h. For coating, the LPS was suspended in PBS and Tox A in 0.1 M sodium carbonate, pH 9.6. Sera were diluted in PBS containing 0.02% Tween 20 and 200 μ L of each dilution were added per well. After the addition of the substrate solution (ABTS, Boehringer-Mannheim, Mannheim, FRG), color was allowed to develop for 10 min. Absorbance at 405 nm was measured with a Titertek Multiscan (Flow Laboratories, Hamden, CT) (22). Titers were expressed as the reciprocal of the highest dilution of serum yielding 0.4 of maximal absorbance. The specificity of the assay was confirmed by measuring crossreactivity to *Escherichia coli* LPS and *Salmonella typhi* Ty21a LPS.

SDS-PAGE and immunoblots. SDS-PAGE was performed by the method of Laemmli (23). A 7.5–15% linear gradient acrylamide gel was used, and 4 μ g of purified PA LPS and 2 μ g of purified Tox A in SDS-sample buffer were applied per well. For immunoblot analysis, the samples were electrophoretically transferred to nitrocellulose sheets as described (24). The sheets were incubated sequentially with serum of CF patients diluted at 1:100 to 1:500, peroxidase-labeled, affinity-purified goat anti-human IgG-M-A (Kirkegaard and Perry, Gaithersburg, MD), and with 4-chloro-1-naphthol substrate (Bio-Rad, Glattbrugg, Switzerland). An identical gel was used for silver staining by the method of Tsai and Frasch (25).

IgG and IgA subclass analysis. For determination of human IgG and IgA subclasses, an ELISA was performed as previously described (26). The following subclass-specific murine MAbs were used: anti-human IgG₁ (clone HP6012), anti-human IgG₃ (HP6010), anti-human IgG₄ (HP6011), anti-human IgA₁ (BAM12), anti-human IgA₂ (M27010) (Unipath Limited, Bedford, England), and anti-human IgG₂ (HP6014) (Sigma Chemicals Co., St. Louis, MO). As a second antibody, a horseradish-peroxidase conjugated goat anti-mouse IgG (Bio-Rad Laboratories, Richmond, CA) was used. After addition of substrate, the OD at 405 nm was determined as described above.

Burn wound model. The murine burn wound sepsis model of Stieritz and Holder (27) was used with slight modification as previously described (19). The mice (in groups of six) received 160 μ g of IgG in a volume of 0.2 mL via the tail vein 24 h before the challenge with strain PA-220, s.c. injected into the burned area. During induction of burns, the animals were carefully anesthetized in an atmosphere of methoxyflurane (19).

Statistical analysis. Antibody titers are expressed as means $\pm$ 1 SD. Differences between the patient groups were tested for significance by the Wilcoxon-Mann-Whitney rank sum test (28).

RESULTS

Serum antibodies. The 10 CF patients without PA colonization/infection showed no detectable antibody titers to PA LPS or Tox A. The 68 CF patients with intermittent ($n = 22$) or chronic ($n = 46$) PA bronchopulmonary colonization/infection had elevated serum antibody titers to LPS of IT-1 in 97%, to IT-2 in 69%, to Habs-3 in 74%, to Habs-4 in 71%, and to Tox A in 94%, whereas antibodies to LPS of IT-3, IT-4, IT-5, IT-6, and IT-7 were found in <5%. In Figure 1, the distribution of the two most frequently detected serum antibody titers—to LPS of IT-1 and to Tox A—are shown for the three patient groups. As listed in Table 1, these antibody titers were significantly higher in

Fig. 2. Study patients were assigned to one of four or five categories for age (a), duration of PA colonization (b), degree of underweight (c), CF score (d), radiographic score (e), and vital capacity (f). The correlations of the mean serum antibody titers to LPS of IT-1, IT-2, Habs-3, Habs-4, and Tox A with these different disease stages are shown. Number of patients in parentheses.

patients chronically colonized compared to those intermittently colonized with PA.

PA cultured and isolated from sputum of six CF patients were serotyped and reflected the common types found for the serum antibody titers: Habs-4 (5×), IT-1 (3×), and Habs-3 (2×).

Graphic correlation of mean serum antibody titers with patient age, duration of PA colonization, degree of underweight, CF score, radiographic score, and vital capacity are shown in Figures 2a-f. Statistical analysis was considered inapplicable (28).

SDS-PAGE and immunoblotting. These techniques confirmed the anti-PA LPS-specific profile of the serum antibodies measured (Fig. 3) by reaction with the O-side chain region of LPS of IT-1, IT-2, and Habs-3.

Subclasses. The results of IgG and IgA subclass analysis of serum antibody titers to LPS of PA IT-1, to PA Tox A, and to tetanus toxin are presented in Table 2. Pooled serum from 10 adolescent CF patients with chronic PA colonization, with similar antibody titers, and with tetanus immunization status according to recommended schedules was compared to pooled serum from 18 healthy adult volunteers before and 28 d after

active immunization with PA IT-1 polysaccharide-tetanus toxoid conjugate vaccine. Anti-IT-1-LPS titers in CF patients were substantially higher for all four IgG subclasses, and also for IgA1, compared to the vaccine response in healthy individuals. Anti-tetanus-toxin titers in CF patients and in unvaccinated volunteers showed similar IgG and IgA subclass distribution.

Burn wound model. Table 3 shows the protection against PA infection in a murine burn wound sepsis model by passive transfer of IgG derived from sera of 10 adolescent CF patients chronically colonized with PA (with similar antibody titers to PA LPS) and from sera of 18 healthy adult volunteers before and 28 d after immunization with PA IT-1 polysaccharide-tetanus toxoid conjugate vaccine. IgG antibodies isolated from CF sera were equally protective compared to those isolated from postimmunization sera, whereas IgG antibodies from preimmunization sera were only slightly more effective than control albumin, explained by preexisting specific antibodies.

DISCUSSION

Numerous studies have demonstrated an inverse correlation between serum antibody titers against a variety of relatively

Fig. 3. SDS-PAGE and immunoblotting technique. Purified LPS derived from PA IT-1 to IT-5 (lanes 1-5), from Habs-3 and Habs-4 (lanes 6 and 7), and from *E. coli* (lane 8) were each subjected to SDS-PAGE (A), gel developed by silver-staining; (B), immunoblot with serum of a CF patient diluted 1:1000, showing bands in the O-side chain region of smooth-type LPS of IT-1, IT-2, and Habs-3.

Table 2. IgG and IgA subclass distribution of antibody titers in pooled serum from CF patients chronically colonized with PA and from healthy individuals before and after PA IT-1 polysaccharide-tetanus toxoid conjugate vaccine

Source of serum pool	Anti-IT-1-LPS titers						Anti-PA-Tox A titers						Antitetanus-toxin titers					
	G ₁	G ₂	G ₃	G ₄	A ₁	A ₂	G ₁	G ₂	G ₃	G ₄	A ₁	A ₂	G ₁	G ₂	G ₃	G ₄	A ₁	A ₂
CF patients (n = 10)	1280	1290	210	120	550	0	260	50	40	60	70	0	80	40	0	40	0	0
Healthy individuals (n = 18)																		
preimmunization	20	30	0	0	0	0	ND*	ND	ND	ND	ND	ND	120	20	0	30	0	0
postimmunization	120	250	0	0	50	0	ND	ND	ND	ND	ND	ND	5120	460	110	1510	20	0

* Not done.

unspecific PA antigens and stage of CF disease (6-14). There is clear support for the hypothesis that the pronounced antibody response to PA antigens has deleterious effects because of immune complex-induced destruction of lung tissue (3-5, 11, 13).

This study reports for the first time results of serum IgG antibodies to LPS of the nine most frequently isolated serotypes of PA in patients with CF. Anti-LPS profile of these antibodies was confirmed by PAGE and immunoblotting techniques. The most common PA types in our CF population are IT-1, IT-2,

Habs-3, and Habs-4. Mean serum antibody titers to LPS of these four serotypes correlated with duration of PA colonization/infection, and with disease severity as reflected by chronologic age, underweight, CF and radiographic scores, and vital capacity. These correlations with the anti-Tox A-titers were clearly less pronounced than those with the serotype-specific antibody responses, which is in certain contrast to other reports (7, 13).

Currently, there is considerable interest in the role of IgG subclasses in CF (29-31). Recently, R. B. Moss *et al.* (31) have

Table 3. Mean LD₅₀ of PA-220 in a murine burn wound sepsis model after passive transfer of IgG derived from sera of CF patients chronically colonized with PA and from healthy individuals before and after PA IT-1 polysaccharide-tetanus toxoid conjugate vaccine

IgG derived from serum of:	Mean LD ₅₀ *	Antibody titers to:		
		LPS PA-220	PA Tox A	Tetanus toxin
Patient CF-6	9.4 × 10 ⁵	900	240	480
Patient CF-9	1 × 10 ⁶	1280	160	420
Patient CF-24	5 × 10 ⁶	980	240	540
CF pool (n = 10)	9.4 × 10 ⁵	1120	160	394
Healthy individuals (n = 18)				
preimmunization	2 × 10 ³	45	ND†	260
postimmunization	9.4 × 10 ⁵	560	ND	1280
Control albumin	3 × 10 ²			

* Groups of six mice were used for each challenge dose.

† Not done.

reported altered serum antibody isotype response to heptavalent LPS of PA in patients with CF. Based on *in vitro* studies (mouse alveolar macrophage phagocytosis of PA) this research group proposed a possible role in opsonic deficiency by increased levels of IgG₄ antibodies which could contribute to impaired pulmonary clearance of PA (30). Our results confirmed that CF patients colonized with PA develop elevated serum levels of all four IgG subclass antibodies to LPS of PA, whereas healthy individuals only respond with IgG₁ and IgG₂ antibodies to PA conjugate vaccine. IgA subclass analysis of serum Anti-LPS-antibodies revealed exclusive IgA₁ response in both CF patients and immunized controls. Brandtzaeg *et al.* (32) have shown that IgA₁ synthesis dominates among immunocytes producing secretory IgA in the upper part of the body (respiratory tract) and IgA₂ in the lower part (gastrointestinal tract).

In a murine burn wound sepsis model, we have previously demonstrated the serotype-specific protective capacity of IgG antibodies to LPS of PA isolated from rabbit hyperimmune sera (19). In contrast, passive transfer of anti-Tox A or antielastase IgG antibodies did not significantly alter the experimental PA infection (19). Sawada *et al.* (33) have confirmed these findings in the same animal model with MAbs produced by mouse hybridoma cells. In this study we found that the IgG antibodies to LPS of PA derived from CF sera were as protective as those derived from postimmunization sera of healthy controls.

In conclusion, our results indicate that anti-LPS antibodies to specific PA-serotypes in serum may be a sensitive measure of severity and prognosis of CF. Longitudinal studies are warranted to confirm this hypothesis. The role of IgG and IgA subclass distribution of antibody response to LPS of PA requires further investigation. Patients with CF show adequate functional immune response to PA with regard to IgG antibodies to LPS of PA. It is possible that vaccination against PA of CF patients who have not yet been colonized with PA could induce protective immunity. Safety and immunogenicity of PA polysaccharide Tox A conjugate vaccines in humans have been satisfactorily documented (34).

Acknowledgments. The authors thank Dr. H. Tschaeppler for evaluation of the chest radiographs, Dr. R. Kraemer for performance of lung function studies, B. Born for technical assistance, and R. Ritzmann for secretarial services.

REFERENCES

- Marks MI 1981 The pathogenesis and treatment of pulmonary infections in patients with cystic fibrosis. *J Pediatr* 98:173-179
- Vasil ML 1986 *Pseudomonas aeruginosa*: biology, mechanisms of virulence, epidemiology. *J Pediatr* 108:800-805
- Hoiby N, Döring G, Schiøtz PO 1987 Pathogenic mechanisms of chronic *Pseudomonas* infections in cystic fibrosis patients. *Antibiot Chemother* 39:60-76
- Pier GB 1985 Pulmonary disease associated with *Pseudomonas aeruginosa* in cystic fibrosis: current status of the host-bacterium interaction. *J Infect Dis* 151:575-580
- Fick RB 1989 Lung humoral response to *Pseudomonas* species. *Eur J Clin Microbiol Infect Dis* 8:29-34
- Döring G, Hoiby N 1983 Longitudinal study of immune response to *Pseudomonas aeruginosa* antigens in cystic fibrosis. *Infect Immun* 42:197-201
- Hollings AE, Granström M, Vasil ML, Wretling B, Strandvik B 1987 Prospective study of serum antibodies to *Pseudomonas aeruginosa* exoproteins in cystic fibrosis. *J Clin Microbiol* 25:1868-1874
- Brett MM, Ghoneim ATM, Littlewood JM, Losowsky MS 1986 Development of enzyme linked immunosorbent assay (ELISA) to detect antibodies to *Pseudomonas aeruginosa* cell surface antigens in sera of patients with cystic fibrosis. *J Clin Pathol* 39:1124-1129
- Brett MM, Ghoneim ATM, Littlewood JM 1987 Serum IgG antibodies in patients with cystic fibrosis with early *Pseudomonas aeruginosa* infection. *Arch Dis Child* 62:357-361
- Hancock RE, Mouat ECA, Speert DP 1984 Quantification and identification of antibodies to outer-membrane proteins of *Pseudomonas aeruginosa* in sera of patients with cystic fibrosis. *J Infect Dis* 149:220-226
- Dasgupta MK, Zuberbuehler P, Abbi A, Harley FL, Brown NE, Lam K, Dossetor JB, Costerton JW 1987 Combined evaluation of circulating immune complexes and antibodies to *Pseudomonas aeruginosa* as an immunologic profile in relation to pulmonary function in cystic fibrosis. *J Clin Immunol* 7:51-58
- Pedersen SS, Espersen F, Hoiby N 1987 Diagnosis of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis by enzyme-linked immunosorbent assay. *J Clin Microbiol* 25:1830-1836
- Moss RB, Hsu Y-P, Lewiston NJ, Curd JG, Milgrom H, Hart S, Dyer B, Larrick JW 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
- Fomsgaard A, Hoiby N, Shand GH, Conrad RS, Galanos C 1988 Longitudinal study of antibody response to lipopolysaccharides during chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis. *Infect Immun* 56:2270-2278
- Schaad UB, Wedgwood-Krucko J, Suter S, Kraemer R 1987 Efficacy of inhaled amikacin as adjunct to intravenous combination therapy (ceftazidime and amikacin) in cystic fibrosis. *J Pediatr* 111:599-605
- Kraemer R, Rüdeberg A, Kläy M, Rossi E 1979 Relationship between clinical conditions, radiographic findings and pulmonary functions in patients with cystic fibrosis. *Helv Paediatr Acta* 34:417-428
- Crispin AR, Norman AP 1974 The systematic evaluation of chest radiograph in cystic fibrosis. *Pediatr Radiol* 2:101-106
- Kraemer R, Meister B, Schaad UB, Rossi E 1983 Reversibility of lung function abnormalities in children with perennial asthma. *J Pediatr* 102:347-350
- Cryz Jr SJ, Fürer E, Germanier R 1983 Protection against *Pseudomonas aeruginosa* infection in a murine burn wound sepsis model by passive transfer of antitoxin A, antielastase, and antilipopolysaccharide. *Infect Immun* 39:1072-1079
- Cryz Jr SJ, Pitt TL, Fürer E, Germanier R 1984 Role of lipopolysaccharide in virulence of *Pseudomonas aeruginosa*. *Infect Immun* 44:508-513
- Liu PV, Matsumoto H, Kusama H, Bergan T 1983 Survey of heat-stable, major somatic antigens of *Pseudomonas aeruginosa*. *Int J Syst Bacteriol* 33:256-264
- Fürer E, Cryz Jr SJ, Dorner F, Nicolet J, Wanner M, Germanier R 1982 Protection against colibacillosis in neonatal piglets by immunization of dams with prochloroenoid. *Infect Immun* 35:887-894
- Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685
- Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76:4350-4354

25. Tsai CM, Frasch CE 1982 A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal Biochem* 119:115-119
26. Cryz SJ, Sadoff JC, Ohman D, Furer E 1988 Characterization of the human immune response to a *Pseudomonas aeruginosa* O-polysaccharide-toxin A conjugate vaccine. *J Lab Clin Med* 111:701-707
27. Stieritz DD, Holder IA 1975 Experimental studies of the pathogenesis of infections due to *Pseudomonas aeruginosa*: description of a burned mouse model. *J Infect Dis* 113:688-691
28. Matthews DE, Farewell VT (eds) 1985 *Using and Understanding Medical Statistics*. Karger, Basel, New York, pp 1-200
29. Fick RB, Olchowski J, Squier SU, Merrill WW, Reynolds HY 1986 Immunoglobulin-G subclasses in cystic fibrosis. *Am Rev Respir Dis* 133:418-422
30. Moss RB 1986 IgG subclasses in respiratory disorders: cystic fibrosis. *Monogr Allergy* 19:202-209
31. Moss RB, Hsu Y-P, Sullivan MM, Lewiston NJ 1986 Altered antibody isotype in cystic fibrosis: possible role in opsonic deficiency. *Pediatr Res* 20:453-459
32. Brandtzaeg P, Kett K, Rognum TO, Söderström R, Björkander J, Söderström T, Petrusson B, Hanson LA 1986 Distribution of mucosal IgA and IgG subclass-producing immunocytes and alterations in various disorders. *Monogr Allergy* 20:179-194
33. Sawada S, Suzuki M, Kawamura T, Fujinaga S, Masuho Y, Tomibe K 1984 Protection against infection with *Pseudomonas aeruginosa* by passive transfer of monoclonal antibodies to lipopolysaccharides and outer membrane proteins. *J Infect Dis* 150:570-576
34. Cryz Jr SJ, Furer E, Cross AS, Wegmann A, Germanier R, Sadoff JC 1987 Safety and immunogenicity of a *Pseudomonas aeruginosa* O-polysaccharide toxin A conjugate vaccine in humans. *J Clin Invest* 80:51-56