Antibody responses induced by long-term vaccination with an octovalent conjugate *Pseudomonas aeruginosa* vaccine in children with cystic fibrosis

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Abstract

We assessed the serological responses over 10 years to repeated immunization of cystic fibrosis (CF) patients with an O-polysaccharide (OPS)–toxin A conjugate vaccine against *Pseudomonas aeruginosa*. A retrospective analysis was performed with sera from 25 vaccinated and 25 unvaccinated children treated at the same CF centre and matched for clinical management, age and gender. Yearly immunization led to sustained elevations of serum immunoglobulin G (IgG) antibody levels to all vaccine components. Eighteen unvaccinated patients but only eight vaccinated ones developed chronic pseudomonal lung infections. Infection rapidly caused further marked elevations of polysaccharide- but not toxin A-specific serum IgG in both immunized and nonimmunized patients, indicating that protection did not depend on the quantity of IgG present. However, qualitative analyses revealed that the protective capacity of specific serum IgG antibodies was linked to high affinity and to specificity for OPS serotypes rather than for lipopolysaccharide core epitopes.

Introduction

Chronic pulmonary infection with *Pseudomonas aeruginosa* is the most important cause of morbidity and mortality in patients with cystic fibrosis (CF), who have impaired immunity at the mucosal level due to high viscosity and salt content of their lung secretions. The body's unsuccessful attempts to eradicate the colonizing bacteria lead to massive inflammation accompanied by lung tissue destruction and loss of function (Konstan, 1998; Lyczak *et al.*, 2002) and accelerate CF lung disease (Kosorok *et al.*, 2001). Effective prophylaxis against *P. aeruginosa* infection by vaccination could improve the management of CF. Many vaccines have been considered (Holder, 2001; Sedlak-Weinstein *et al.*, 2005) and in addition to whole-cell vaccines (Cripps *et al.*, 2006) various specific pathogenicity factors of *P. aeruginosa* have been targeted, such as outer membrane proteins (OMPs) (Von Specht *et al.*, 1996; Jang *et al.*, 1999), pili (Sato *et al.*, 1988), flagellae (Holder *et al.*, 1982) and secreted products (Matsumoto *et al.*, 1998). More recently, translocation proteins (PcrV) of type III secretion systems (Sawa *et al.*, 1999) have been used. Another possible vaccine target is surface lipopolysaccharide, although only high-affinity antibodies are functional and will confer protection (Lang *et al.*, 1995). We have developed an octovalent O-polysaccharide (OPS) – toxin A conjugate vaccine that achieves optimal induction of anti-*P. aeruginosa* lipopolysaccharide antibodies. In 1989 a group of young children with CF at the Children's Hospital of the University Hospital of Bern, Switzerland, who had not yet become infected with

P. aeruginosa, was treated with the vaccine to study its safety and immunogenicity (Schaad *et al*., 1991). Following initial vaccination, annual boosters were given except at year 2, and interim results were reported after 3 (Cryz *et al*., 1994) and 4 (Lang *et al*., 1995) years of follow-up. Finally, a retrospective review of microbiology and clinical data for 25 of these patients spanning a 10-year period has recently been presented (Lang *et al*., 2004). The results indicated a good safety profile for long-term use. Fewer vaccinated patients became chronically infected with *P. aeruginosa* compared with the control group. This was associated with better preservation of lung function and improved weight gain. Here we describe the humoural immune responses of these patients during the 10-year period.

Materials and methods

Vaccine

The octovalent conjugate vaccine used has been described in detail elsewhere (Schaad *et al*., 1991; Cryz *et al*., 1994, 1987; Lang *et al*., 1995, 2004). It comprises OPS from eight lipopolysaccharide serotypes of *P. aeruginosa* [International Antigenic Typing Scheme (IATS) 1, 3, 4, 5, 6, 10, 11 and 16] covalently coupled to toxin A of *P. aeruginosa*. Initial inoculations were given at 0, 2 and 12 months, and annual booster doses commenced from the third year in all patients, i.e. also in patients who became chronically infected.

Patients and study design

Twenty-five vaccinated CF patients were compared with 25 controls drawn from the same CF centre, of whom 21 were pairs matched on age and gender. The institution's independent ethics committee approved the study, and patients or their parents as appropriate gave informed consent. The demographics and clinical management of the groups have been fully reported (Lang *et al*., 2004). In summary, the mean ages at enrolment and follow-up in the vaccinated group (11 females, 14 males) were 7.4 years (1.7–15.5 years) and 17.2 years (13.7–24.6 years). In the control group (13 females, 12 males) the mean ages were 6.4 years (range 1.3–22.1 years) and 16.5 years (9.7–33.6 years), respectively. Patients in both groups received the same clinical management in all respects other than immunization. Antibiotic treatment was given for first and subsequent *P. aeruginosa* infections and for lung infections with other organisms according to international protocols, including the 2000 European consensus document (Doring *et al*., 2000).

Mean observation times in the vaccinated and control patients were 9.8 and 10.1 years, respectively. Sputum and throat swabs were obtained at each follow-up visit and cultured for various bacterial species including *P. aeruginosa*. Chronic *P. aeruginosa* lung infection was said to have occurred when *Pseudomonas* was consistently cultured from sputum and/or throat swabs during a period of at least 6 months, and more than two precipitating antibodies against *P. aeruginosa* were detected, as indicated by multiple precipitin lines. Serotyping of *P. aeruginosa* strains from infected patients was performed up to year 4 of follow-up (Lang *et al*., 1995). Thereafter, no information concerning infecting serotypes is available as data were collected retrospectively, and serotyping was not performed routinely in the study centre.

A total of 774 sera, including baseline samples, was collected from immunized and control patients, and analysed over the 10-year observation period. During the first 4 years serum samples were collected and analysed at intervals of not more than 6 months, and data were recorded prospectively. After this, samples were taken as clinically indicated, but at least at yearly intervals for most patients. These test results were collected retrospectively at the end of the study period. Consequently, data were not available for all patients at all time points.

Measurement of specific serum IgG by ELISA

Serum immunoglobulin G (IgG) antibodies against all vaccine components (*P. aeruginosa* IATS lipopolysaccharide serotypes 1, 3, 4, 5, 6, 10, 11, 16 and *P. aeruginosa* toxin A) were measured by enzyme-linked immunosorbent assay (ELISA). *Pseudomonas aeruginosa* lipopolysaccharide antigen ($5\,\mu g\,mL^{-1}$) was bound to polystyrene microtitre plates (NUNC Maxisorp, Roskilde, Denmark) by methylated human serum albumin. After washing, serially diluted sera and standard reference serum were incubated for 2 h at ambient temperature. Plates were washed, and incubated with alkaline phosphatase-labelled goat antihuman IgG antibody (Sigma, St Louis, MO, USA) for 1.5 h. After further washing, plates were developed with 4-nitrophenylphosphate (Merck, Darmstadt, Germany), and optical density (OD) at 405 nm was measured using a Spectromax ELISA-plate reader (Paul Bucher AG, Basel, Switzerland). OD values were transformed to micrograms per millilitre with SoftMax Pro software version 3.1.1 (Molecular Devices, Sunnyvale, CA, USA) using a standard curve of a human reference serum. All assays were validated according to good manufacturing practice standards and showed $<10\%$ intra- and interassay variation.

Affinity determination

Affinity constants of serum IgG antibodies specific for *P. aeruginosa* lipopolysaccharide serotype IATS-6 were measured by inhibition ELISA as described by Bruderer *et al*. (1992). This serotype was selected as a surrogate measure for affinity of antipolysaccharide antibodies as it had been used in past studies (Lang *et al*., 1995) and so comparative data

were available. They were defined as the reciprocal antigen concentration (in moles per litre) resulting in 50% inhibition of antibody binding. Calculation was according to the method described by Reed and Muench (1938). The last available serum sample for all patients was used.

Cross-inhibition ELISA

To determine epitope specificity, cross-inhibition ELISA was performed by a method essentially identical to that described above for affinity determination. Either purified lipopolysaccharide of *P. aeruginosa* serotypes IATS-6 and IATS-3 or purified OPS, isolated by cleavage of the lipid A and core portions from the OPS through mild acid hydrolysis, was used for inhibition (Cryz *et al.*, 1987).

Statistical analysis

Because some data were collected retrospectively, and the numbers and timing of observations varied between patients, results are generally presented descriptively. Where outcomes have been compared between the treatment groups, the two-tailed, unpaired rather than the paired *t*-test has been employed, as not all vaccinated patients were fully matched with controls.

Results

Incidence of infection

The clinical outcomes after 10 years of follow-up, including infection rates, have been reported in detail recently (Lang *et al.*, 2004). Briefly, of the 25 patients receiving the vaccine, 15 remained free of chronic *Pseudomonas* infection throughout the entire observation period, two had transient infection and eight developed chronic infection (defined as described above). Up to year 4 of follow-up the serotypes of the infecting *Pseudomonas* strains were those contained in the vaccine, indicating that no strain replacement had occurred. In the nonimmunized control group of 25 patients, only seven remained free of chronic infection and 18 became infected.

Serum IgG antibodies

Table 1 shows the geometric mean concentrations of specific serum IgG antibodies at specified time points for the following pooled groups of patients: immunized and remaining free from chronic infection with *P. aeruginosa*; immunized but acquiring chronic infection; nonimmunized and infection free; and non-immunized and becoming infected.

Initial immunization (months 0 and 2) led to the induction of specific serum IgG antibodies against all nine antigens (data not shown). These specific antipolysaccharide antibodies tended to wane rapidly after immunization.

Table 1. Specific serum IgG antibody concentrations [geometric mean (range), $\mu g\,mL^{-1}$] in various patient groups

	Immunized, noninfected ($n = 17$)		Immunized, infected ($n = 8$)			Nonimmunized, noninfected ($n = 7$)			Nonimmunized, infected ($n = 18$)		
Serotype	Pre ($n = 14$)*	Month 120 ($n = 12$)	Pre ($n = 7$)	Last before infection ($n = 8$)	Month 120 ($n = 7$)	Pre ($n = 4$)	Month 120 ($n = 6$)	Pre ($n = 8$)	Last before infection ($n = 10$)	Month 120 ($n = 13$)
IATS-1	4.0 (0.0–8.7)	18.8 (8.9–81.6)	4.2 (0.0–9.2)	9.2 (4.0–23.1)	85.1 (31.2–415.5)	3.8 (1.7–13.9)	6.7 (1.8–31.9)	7.3 (2.1–35.6)	5.6 (2.6–15.7)	69.4 (9.2–417.0)
IATS-3	1.7 (0.0–6.2)	9.0 (4.7–226.1)	1.5 (0.6–2.6)	8.5 (3.4–30.3)	157.7 (34.4–1428.4)	2.4 (0.8–9.8)	3.6 (0.8–52.0)	2.9 (0.7–9.4)	3.5 (0.4–14.1)	85.2 (1.9–809.1)
IATS-4	4.5 (0.0–13.1)	14.5 (10.6–124.0)	4.9 (2.0–16.6)	14.4 (6.7–56.3)	196.4 (19.7–4265.8)	4.4 (1.0–19.2)	4.0 (0.5–64.1)	6.4 (0.6–68.7)	8.4(0.9–68.7)	189.5 (6.9–1288 8)
IATS-5	2.8 (0.0–20.6)	29.0 (11.6–110.6)	3.1 (1.9–6.1)	18.6 (4.2–212.6)	143.0 (59.0–416.9)	5.2 (0.0–14.0)	5.6 (0.0–35.4)	8.4 (0.0–43.1)	15.9 (3.0–138.5)	62.6 (10.5–291.1)
IATS-6	3.0 (0.0–7.3)	26.5 (9.9–243.3)	3.9 (0.0–24.4)	16.9 (3.6–247.8)	164.0 (64.2–336.8)	3.6 (0.0–7.9)	5.0 (0.0–13.1)	4.3 (0.0–9.9)	5.0 (1.9–13.2)	74.7 (5.7–276.1)
IATS-10	1.0 (0.0–3.8)	7.4 (4.7–53.2)	8.1 (0.0–39.6)	6.9 (2.3–17.1)	62.1 (17.8–229.6)	1.7 (0.0–3.2)	3.2 (1.7–7.8)	1.7 (0.0–6.5)	2.5 (0.6–6.5)	57.5 (6.0–391.6)
IATS-11	3.8 (0.0–12.3)	15.3 (13.6–137.7)	2.0 (0.5–5.4)	14.6 (2.3–156.0)	134.6 (30.5–400.5)	3.4 (0.9–6.8)	9.6 (2.2–23.6)	3.7 (0.5–28.9)	8.8 (0.5–308.0)	87.0 (3.9–491.4
IATS-16	4.1(0.0–29.7)	25.4 (9.7–153.5)	6.1 (1.5–20.8)	21.4(5.8–91.9)	103.4 (39.7–246.2)	4.5 (0.0–12.5)	5.8 (2.7–19.7)	7.4 (0.0–93.4)	14.2 (2.3–133.8)	43.5 (18.4–93.0)
Toxin A	3.3 (0.0–10.8)	45.3 (27.5–140.9)	3.5 (1.9–10.7)	101.1 (22.9–688.1)	86.9 (34.5–279.9)	2.5 (0.0–5.4)	3.0 (1.1–7.6)	6.9 (0.0–59.8)	11.6 (0.0–63.4)	42.9 (10.2–233.9)

*Sera were not available for all patients for all time points.
IATS, International Antigenic Typing Scheme.

However, they could be repeatedly reinduced by yearly booster immunizations (data not shown). Using this immunization schedule, relatively low but significant mean polysaccharide-specific serum IgG levels of 7.4–29.0 $\mu g\,mL^{-1}$ were maintained over the 10-year period in the immunized, noninfected group (Table 1). Table 1 also shows the serum IgG levels in the eight immunized patients who became chronically infected during the observation period. Prevaccination values and the last samples taken before the time at which each individual acquired the infection demonstrated similar antipolysaccharide antibody induction as in immunized, noninfected patients. However, after the onset of chronic infection further rapid induction of large amounts of IgG was observed, often reaching levels of $>100\,\mu g\,mL^{-1}$. By contrast, infection did not typically lead to elevation of antitoxin-A-specific IgG concentrations, but rather to a decline. Nonimmunized patients generally had similarly low starting levels of antibodies, and these had changed little in the month 120 samples in those remaining noninfected and in the last sample before the onset of chronic infection in the others. As observed in immunized patients, upon chronic pseudomonal infection IgG levels rose rapidly to high values. The final serum samples showed similar high values to those in infected immunized cases.

Affinity of IATS 6-specific serum IgG

We qualitatively investigated vaccine- vs. infection-induced specific antibodies to attempt to determine which parameters correlated with protection. We first measured mean affinity constants of anti-IATS-6 IgG antibody by inhibition ELISA in individual serum samples. This serotype was chosen owing to the availability of historical data for the same antigen (Lang *et al.*, 1995), and we assumed that similar results would be obtained with other polysaccharide serotypes contained in the vaccine. Values were compared in immunized and nonimmunized patients between those who remained noninfected and those who subsequently acquired a chronic infection (using the last available serum sample before the onset of infection) (Fig. 1). The mean affinity constant in immunized patients who remained free of chronic infection was more than 10 times higher than in immunized patients who became chronically infected ($1.76 \times 10^8\,M^{-1}$ vs. $1.60 \times 10^7\,M^{-1}$, $P = 0.010$ by two-tailed, unpaired *t*-test). It was also much higher than in nonimmunized patients who became chronically infected ($2.72 \times 10^7\,M^{-1}$, $P = 0.016$).

Epitope specificity of serum IgG antibodies

Next, the epitope specificity of vaccine- vs. infection-induced IgG was analysed by cross-inhibition ELISA in four immunized patients who acquired a chronic infection. 'Early' serum samples taken before the onset of infection, presumed to contain mainly vaccine-induced IgG antibodies, were compared with 'late' ones collected after chronic infection had started, containing mainly infection-induced antibodies. A representative example of these analyses (patient 104) is shown in Fig. 2a, using samples taken at 13.9 months after first immunization (early) and at 128.5 months after first immunization, i.e. after the onset of chronic infection (late). At this time point IgG not only to IATS-3 serotype but to all serotypes was increased. This phenomenon was observed in all patients upon chronic infection (data not shown). As shown in Fig. 2b, anti-IATS-3 antibodies from the 'early' serum sample were strongly inhibited by IATS-3 OPS and lipopolysaccharide, but not by OPS and lipopolysaccharide preparations of another serotype (IATS-6). This indicated that antibodies contained in this serum sample were specific for a structure exclusively present on the IATS-3 polysaccharide, presumably the OPS portion. By contrast, anti-IATS-3 antibodies in the 'late'

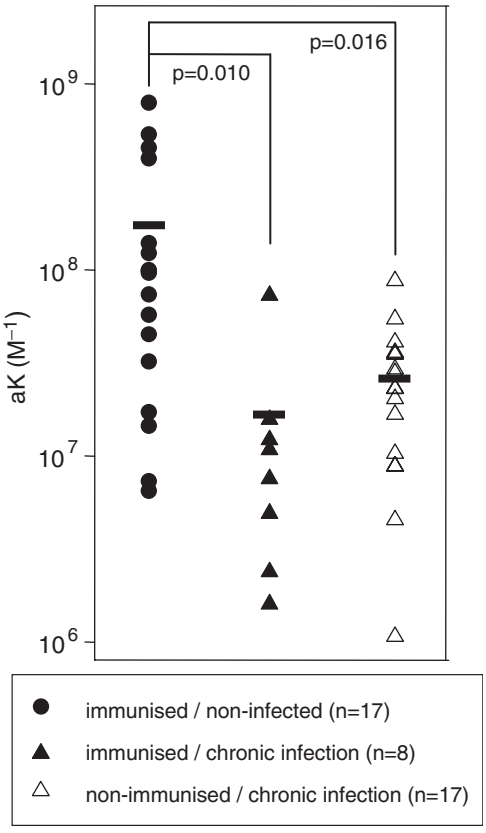

Fig. 1. Antibody affinity constants to *Pseudomonas aeruginosa* serotype IATS-6 for three different patient subgroups. The affinity of IATS-6-specific serum IgG antibodies in individual serum samples was determined by inhibition ELISA in various patient groups as indicated. Each point represents the antibody affinity constant for an individual patient at different time points. The bar shows the mean antibody affinity constant of each patient subgroup.

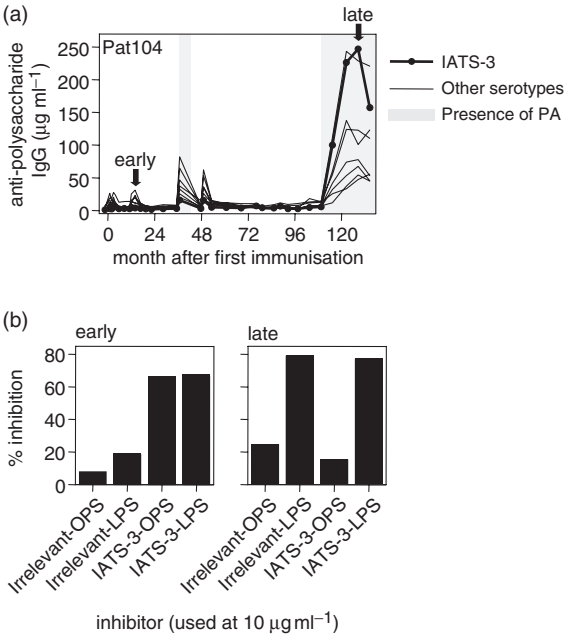

Fig. 2. Specificity of vaccine- vs. infection-induced serum IgG antibodies in a chronically infected patient (104). (a) Timing of samples. Arrows indicate serum samples used for analysis of epitope specificity. The shaded bars indicate periods of presence of *Pseudomonas aeruginosa*. The 'early' sample was collected before occurrence of any infection, and is therefore assumed to contain vaccine-induced antibodies only. The 'late' sample was collected after the onset of chronic pseudomonal lung infection and is assumed to contain mainly infection-induced antibodies. (b) Antibody inhibition. Epitope specificity of vaccine- and infection-induced antibodies was determined by cross-inhibition ELISA. Binding of serum IgG antibodies to solid-phase IATS-3 was inhibited with OPS and LPS preparations of IATS-3 and an unrelated (IATS-6) serotype. Results for one patient are shown; sera of four patients were analysed in total.

serum sample were no longer inhibited by IATS-3 OPS but were still inhibited by IATS-3 lipopolysaccharide. Additionally, IATS-6 lipopolysaccharide inhibited antibody binding to IATS-3, indicating that these antibodies were specific for structures distinct from OPS and shared by different lipopolysaccharide types. Similar results were obtained with sera from the other patients (data not shown). These results demonstrate that vaccine-induced antibodies are monospecific for the different serotypes (OPS), whereas infection-induced antibodies recognize cross-reactive or common epitopes within the core region of lipopolysaccharide.

Discussion

Pseudomonas aeruginosa is a classical opportunistic pathogen that does not harm normal healthy individuals after natural exposure. It only poses a threat to hosts with impaired immunity, who are unable to mount effective immune responses to naturally acquired *Pseudomonas* that would lead to elimination of the pathogen. In CF patients, who are otherwise immunocompetent, this is caused by impairment of the physiological integrity of the mucus as a result of its high viscosity and salt content, making local immune responses rather ineffective. This is reflected in the high rate at which early infection subsequently becomes chronic.

In this evaluation of a *Pseudomonas* conjugate vaccine we used yearly immunizations to maintain a prophylactic level of specific IgG antibodies in the blood. This unusually frequent immunization schedule was chosen because conjugate vaccine-induced antipolysaccharide antibodies tend to wane rapidly (Fairley *et al.*, 1996; Richmond *et al.*, 2001). The same phenomenon was observed with our vaccine (data not shown), although yearly immunization resulted in relatively low but sustained levels of vaccine-specific serum IgG antibodies. Moreover, common conjugate vaccines such as those against *Haemophilus influenzae*, *Streptococcus pneumoniae* or *Neisseria meningitidis* are not used repeatedly but only until the immune system of the child has matured sufficiently to be able to produce functional antipolysaccharide antibodies on its own. By contrast, antipseudomonal prophylaxis in CF patients needs to be maintained *ad infinitum*.

With the onset of infection a massive stimulation of specific serum IgG levels was observed in both immunized and nonimmunized patients, as has been described by others (Fomsgaard *et al.*, 1988; Tosi *et al.*, 1995). The ability to stimulate high levels of lipopolysaccharide-specific antibody with low effector potential might be regarded as a virulence factor of *Pseudomonas*. As a consequence it is not practicable to use the serum concentration of specific IgG antibodies as a surrogate marker for protection from a *Pseudomonas* vaccine, unlike other conjugate vaccines against bacterial pathogens such as *H. influenzae* (Käyhty *et al.*, 1983), *S. pneumoniae* (Saeland *et al.*, 2000) or *N. meningitidis* (Sikkema *et al.*, 2000).

The occurrence of chronic infection in the presence of very high IgG levels clearly demonstrates that protection mediated by specific IgG antibodies is not correlated to their quantity in serum. There appeared to be no differences in the levels of specific antibodies in the vaccinated patients who succumbed to chronic infection and those who did not. However, earlier analyses in the same patient group (Bruderer *et al.*, 1992; Lang *et al.*, 1995) suggested a relationship between affinity and protection. This concept has been confirmed for other *P. aeruginosa* antigens (Ciofu *et al.*, 1999) and different bacterial pathogens (Hetherington & Lepow, 1992; Romero-Steiner *et al.*, 1999). Our present data corroborate these findings, with the mean affinity constant of uninfected, immunized patients being about 1 log higher than for infected patients, whether immunized or not.

However, the large range of affinities found in different patients implies that additional mechanisms may protect against infection.

Pseudomonal lung infections in CF patients invariably occur with a single clonal variant (Breitenstein *et al*., 1997; Tummler *et al*., 1997; Asboe *et al*., 1998). Chronic infection with *Pseudomonas* led to a rise of antipolysaccharide IgG against epitopes present in all serotypes, and not just a single one corresponding to the infecting strain. We hypothesize that, with the well-known change of *P. aeruginosa* in the CF lung from smooth to rough lipopolysaccharide phenotype, antibodies directed to the OPS portion of the surface lipopolysaccharide might be replaced by cross-reactive antibodies against the conserved lipopolysaccharide core region. This theory was supported by our cross-inhibition experiments.

Conclusions

Yearly immunizations with an octovalent OPS – toxin A conjugate *P. aeruginosa* vaccine in a cohort of 25 CF patients over 10 years resulted in maintenance of elevated levels of specific serum IgG antibodies against the vaccine components. The vaccine-induced IgG levels were low compared with infection-induced IgG levels, but qualitative characterization showed that affinity and epitope specificity rather than the quantity of the antibodies mediates the protective effect of the vaccine. Effective prophylaxis against chronic *P. aeruginosa* infection of CF patients is an important unmet medical need. Our 10-year experience suggests that this vaccine is immunogenic, clinically effective and has a good safety profile (Lang *et al*., 2004). A multicentre, double-blind, placebo-controlled phase III clinical trial is currently in progress to confirm these preliminary results.

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References

Asboe D, Gant V, Aucken HM, Moore DA, Umasankar S, Bingham JS, Kaufmann ME & Pitt TL (1998) Persistence of *Pseudomonas aeruginosa* strains in respiratory infection in AIDS patients. *AIDS* **12**: 1771–1775.

Breitenstein S, Walter S, Bosshammer J, Romling U & Tummler B (1997) Direct sputum analysis of *Pseudomonas aeruginosa* macrorestriction fragment genotypes in patients with cystic fibrosis. *Med Microbiol Immunol (Berlin)* **186**: 93–99.

Bruderer U, Cryz SJ Jr, Schaad UB, Deusinger M, Que JU & Lang AB (1992) Affinity constant of naturally acquired and vaccine-induced anti-*Pseudomonas aeruginosa* antibodies in healthy adults and cystic fibrosis patients. *J Infect Dis* **166**: 344–349.

Ciofu O, Petersen TD, Jensen P & Hoiby N (1999) Avidity of anti-*P aeruginosa* antibodies during chronic infection in patients with cystic fibrosis. *Thorax* **54**: 141–144.

Cripps AW, Peek K, Dunkley M, Vento K, Marjason JK, McIntyre ME, Sizer P, Croft D & Sedlak-Weinstein L (2006) Safety and immunogenicity of an oral inactivated whole-cell *Pseudomonas aeruginosa* vaccine administered to healthy human subjects. *Infect Immun* **74**: 968–974.

Cryz SJ Jr, Fuerer 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.

Cryz SJ Jr, Wedgewood J, Lang AB, Ruedeberg A, Que JU, Fuerer E & Schaad UB (1994) Immunization of noncolonized cystic fibrosis patients against *Pseudomonas aeruginosa*. *J Infect Dis* **169**: 1159–1162.

Doring G, Conway SP, Heijerman HG, Hodson ME, Høiby N, Smythe A & Touw DJ (2000) Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *Eur Respir J* **16**: 749–767.

Fairley CK, Begg N, Borrow R, Fox AJ, Jones DM & Cartwright K (1996) Conjugate meningococcal serogroup A and C vaccine: reactogenicity and immunogenicity in United Kingdom infants. *J Infect Dis* **174**: 1360–1363.

Fomsgaard A, Hoiby N, Shand GH, Conrad RS & Galanos C (1988) Longitudinal study of antibody response to lipopolysaccharides during chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. *Infect Immun* **27**: 2270–2278.

Hetherington SV & Lepow ML (1992) Correlation between antibody affinity and serum bactericidal activity in infants. *J Infect Dis* **165**: 753–756.

Holder IA (2001) Pseudomonas vaccination and immunotherapy: an overview. *J Burn Care Rehabil* **22**: 311–320.

Holder IA, Wheeler R & Montie TC (1982) Flagella preparations of *Pseudomonas aeruginosa*: animal protection studies. *Infect Immun* **35**: 276–280.

Jang IJ, Kim IS, Park WJ, *et al*. (1999) Human immune response to a *Pseudomonas aeruginosa* outer membrane protein vaccine. *Vaccine* **17**: 158–168.

Konstan MW (1998) Therapies aimed at airway inflammation in cystic fibrosis. *Clin Chest Med* **67**: 239–247.

Kosorok MR, Zeng L, West SE, Rock MJ, Splaingard ML, Laxova A, Green CG, Collins J & Farrell PM (2001) Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. *Pediatr Pulmonol* **32**: 277–287.

Käyhty H, Peltola H, Karanko V & Mäkela PH (1983) The protective level of serum antibodies to capsular polysaccharide of *Haemophilus influenzae* type B. *J Infect Dis* **147**: 1100.

Lang AB, Schaad UB, Ruedeberg A, Wedgwood J, Que JU, Fuerer E & Cryz SJ Jr (1995) Effect of high-affinity anti-*Pseudomonas aeruginosa* lipopolysaccharide antibodies induced by immunization on the rate of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. *J Pediatr* **127**: 711–717.

Lang AB, Rüdeberg A, Schöni MH, Que JU, Fürer E & Schaad UB (2004) Vaccination of cystic fibrosis patients against *Pseudomonas aeruginosa* reduces the proportion of patients infected and delays time to infection. *Pediatr Infect Dis J* **23**: 504–510.

Lyczak JB, Cannon CL & Pier GB (2002) Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* **15**: 194–222.

Matsumoto T, Tateda K, Furuya N, Miyazaki S, Ohno A, Ishii Y, Hirakata Y & Yamaguchi K (1998) Efficacies of alkaline protease, elastase and exotoxin A toxoid vaccines against gut-derived *Pseudomonas* sepsis in mice. *J Med Microbiol* **47**: 303–308.

Reed LJ & Muench HA (1938) A simple method of estimating fifty per cent endpoint. *Am J Hyg* **27**: 493–497.

Richmond P, Borrow R, Findlow J, Martin S, Thornton C, Cartwright K & Miller E (2001) Evaluation of De-O-acetylated meningococcal C polysaccharide-tetanus toxoid conjugate vaccine in infancy: reactogenicity, immunogenicity, immunologic priming, and bactericidal activity against O-acetylated and De-O-acetylated serogroup C strains. *Infect Immun* **69**: 2378–2382.

Romero-Steiner S, Musher DM, Cetron MS, Pais LB, Groover JE, Fiore AE, Plikaytis BD & Carlone GM (1999) Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. *Clin Infect Dis* **29**: 281–288.

Saeland E, Vidarsson G & Jonsdottir I (2000) Pneumococcal pneumonia and bacteremia model in mice for the analysis of protective antibodies. *Microb Pathog* **29**: 81–91.

Sato H, Okinaga K & Saito H (1988) Role of pili in the pathogenesis of *Pseudomonas aeruginosa* burn infections. *Microbiol Immunol* **32**: 131–139.

Sawa T, Yahr TL, Ohara M, Kurahashi K, Gropper MA, Wiener-Kronish JP & Frank DW (1999) Active and passive immunization with the *Pseudomonas* V antigen protects against type III intoxication and lung injury. *Nature Med* **5**: 392–398.

Schaad UB, Lang AB, Wedgwood J, Ruedeberg A, Que JU, Fuerer E & Cryz SJ Jr (1991) Safety and immunogenicity of *Pseudomonas aeruginosa* conjugate A vaccine in cystic fibrosis. *Lancet* **338**: 1236–1237.

Sedlak-Weinstein E, Cripps AW, Kyd JM & Foxwell AR (2005) *Pseudomonas aeruginosa*: the potential to immunize against infection. *Expert Opin Biol Therap* **5**: 967–982.

Sikkema DJ, Friedman KE, Corsaro B, Kimura A, Hildreth SW, Madore DV & Quataert SA (2000) Relationship between serum bactericidal activity and serogroup-specific immunoglobulin G concentration for adults, toddlers, and infants immunized with neisseria meningitidis serogroup C vaccines. *Clin Diagn Lab Immunol* **7**: 764–768.

Tosi MF, Zakem-Cloud H, Demko CA, Schreiber JR, Stern RC, Konstan MW & Berger M (1995) Cross-sectional and longitudinal studies of naturally occurring antibodies to *Pseudomonas aeruginosa* in cystic fibrosis indicate absence of antibody-mediated protection and decline in opsonic quality after infection. *J Infect Dis* **172**: 453–461.

Tummler B, Bosshammer J, Breitenstein S, *et al.* (1997) Infections with *Pseudomonas aeruginosa* in patients with cystic fibrosis. *Behring Inst Mitt* **98**: 249–255.

Von Specht BU, Lucking HC, Blum B, Schmitt A, Hungere KD & Domdey H (1996) Safety and immunogenicity of a *Pseudomonas aeruginosa* outer membrane protein I vaccine in human volunteers. *Vaccine* **14**: 1111–1117.