Safety and Immunogenicity of an Oral Inactivated Whole-Cell *Pseudomonas aeruginosa* Vaccine Administered to Healthy Human Subjects

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This study examines the safety and immunogenicity of an oral, whole-cell *Pseudomonas aeruginosa* vaccine administered to healthy volunteers. Thirty subjects received an oral dose of Pseudostat in two timed, measured doses with serological follow-up to 56 days postvaccination. Following vaccination, several individuals were identified as antibody responders for all three immunoglobulin (Ig) isotypes tested, specifically against whole-cell *P. aeruginosa* extract and outer membrane proteins F and I. The mean pooled lipopolysaccharide antigen-specific IgA showed the most significant and constant increases in titer postdose, with a similar increase in titer for whole-cell *P. aeruginosa* extract-specific IgA. The results demonstrated an increased phagocytic ability of the selected macrophage cell line in post vaccination sera. Furthermore a significant increase in intracellular macrophage killing of opsonized *P. aeruginosa* was also demonstrated (82% on day 14 postdose) in the presence of the postvaccination sera. The safety component of the study did not show any vaccine-attributable adverse effects in any of the subjects, as documented by clinical evidence, hematology, and biochemistry profiles. We conclude that Pseudostat is safe and immunogenic in humans at this dose and that further studies to determine the appropriate dosage and efficacy are needed. In our study, we have shown that the most significant and sustained responses to oral vaccination in human adult volunteers were serum IgA levels and that pooled sera collected postimmunization have an increased capacity to promote opsonophagocytic killing of *P. aeruginosa*.

*Pseudomonas aeruginosa* is a gram-negative bacterium with a ubiquitous distribution within the biosphere. In the compromised host, it is capable of establishing opportunistic infections, and this is particularly common in subjects with lung dysfunction. *P. aeruginosa* is particularly well adapted to the conditions found in the lungs of cystic fibrosis (CF) patients, where a defective chloride channel transport protein results in an increased viscosity of secretions, making it difficult to clear airway mucous. Colonization takes place at an early age, often in the absence of any overt clinical presentation or culture-positive sputum and throat swabs (3). Because the bacteria are not effectively eradicated from the clinical presentation or culture-positive sputum and throat swabs, superoxide radicals, and inflammatory mediators contribute to the subsequent destruction of normal lung tissue. The major antigen of immune complexes in the sputum of CF patients has been shown to be LPS (17). Outer membrane protein F (OprF) and outer membrane protein H2 (OprH2) in particular have been shown to induce strong antibody activity, while OprI, OprF, and OprH2 are highly conserved in *P. aeruginosa* (31, 33).

Current therapies with antibiotics are targeted at controlling bacterial load of *P. aeruginosa* and other bacteria. These frequently fail to adequately clear established infections, while low antibiotic concentrations in the airways are ineffective and may lead to the development of resistant bacterial strains. A vaccine which could prevent or delay initial colonization with *P. aeruginosa* in the lungs may have a positive impact on CF patients and contribute to improvement in quality of life and survival in these patients. In addition, it is also feasible that immunization may reduce bacterial loads in patients who have become chronically colonized with *P. aeruginosa*. Vaccine candidates for *P. aeruginosa* have been under study for some 30 years or more, but progress has been slow (9). The potential to vaccinate against *P. aeruginosa* infection has been recently reviewed, and a number of exciting opportunities have been identified including mucosal immunization (27). Most studies have focused on burn patients and CF patients, and many have not progressed beyond initial proof-of-concept stages.
A Cochrane review in 1999 (16) concluded that there was a paucity of randomized clinical trials assessing the effectiveness of vaccination against *P. aeruginosa* in CF patients. The only trial to meet their inclusion criteria was one evaluating a blended LPS administered to children, which showed no clinical benefit at the 10-year follow-up. There was also a suggestion that the vaccine may have been detrimental, with the immunized group appearing to have more severe pulmonary exacerbations than the control group (18). Although this does not preclude a vaccine approach to *P. aeruginosa* infection in the management of CF, it may have added to the reluctance and slow progress in developing a vaccine.

This study reports on a phase 1 safety and immunogenicity study using an oral inactivated whole-cell *P. aeruginosa* vaccine administered to healthy volunteers. The vaccine has previously been shown to protect against acute *P. aeruginosa* challenges to the lungs of rodents (2, 7) and may be suitable for the development of an oral formulation for CF infants to prevent or delay colonization by *P. aeruginosa* (7). Successful mucosal and systemic immune stimulation mediated via gastrointestinally associated lymphoid tissue would allow for the development of oral vaccines to be used as a prophylactic and therapeutic tool against chronic *P. aeruginosa* infections.

### MATERIALS AND METHODS

#### Clinical trial rationale

The aim of the study was to assess the safety of Pseudostat as an oral vaccine in humans and to obtain preliminary information on antibody responses to *P. aeruginosa* following oral vaccine administration. For the safety trial, treatment was selected as the lowest scaled-up by-weight dose derived from mice studies which had induced a protective response against a *P. aeruginosa* lung challenge and statistically significant increases in serum *P. aeruginosa*-specific antibody titers. Based on animal data, a “priming” and “stimulatory” dose regime was chosen as it could produce a greater response than a single dose.

#### Study design

This was a single-center, open-label, phase 1 study to assess the safety and immune response to Pseudostat, an oral preparation of whole-cell, formaldehyde-inactivated *P. aeruginosa*, in healthy volunteers. Thirty healthy subjects between the ages of 18 and 50 years who met the inclusion criteria were recruited for the study. Subjects were excluded if they had any clinically relevant medical condition including a past history of *Pseudomonas* infections requiring medical treatment, a history of alcohol or drug abuse, any allergic sensitivity or intolerance to vaccines, and impaired oral absorption. They were also excluded if they were immunocompromised, had participated in any study during the last 2 months, were pregnant, or were of childbearing age and had not been taking a combined oral contraceptive pill for at least 3 months.

#### Vaccination and safety

The Pseudostat vaccine consisted of enteric-coated hard gelatin capsules, each containing 150 mg of lyophilized, formaldehyde-inactivated *P. aeruginosa* strain 385, serotype 2, phase type 21/44/109/110C/12/4, which was equivalent to 2 × 10^11* bacteria per capsule. The vaccine was manufactured for the trial by Boehringer-Ingelheim. Strain 385 is a clinical isolate of a mucoid phenotype. In extensive preclinical animal experiments, immunization with this strain gave optimal and reproducible responses, and immune protection associated lymphoid tissue would allow for the development of oral vaccines to be used as a prophylactic and therapeutic tool against chronic *P. aeruginosa* infections.

#### Determination of antibodies to whole-cell extract of *P. aeruginosa* and outer membrane proteins (OppR and OppI)

Enzyme-linked immunosorbent assay (ELISAs) were developed and validated for measuring antibody responses to whole-cell *P. aeruginosa* extract and outer membrane proteins OppR and OppI. Microtiter plates were coated with 100 μl of a 10 μg/ml solution of either soluble whole-cell *P. aeruginosa* extract or recombinant His-tagged OppR or OppI in 50 mM sodium bicarbonate buffer at pH 9.6. Plates were then blocked with 0.1% (vol/vol) Tween 20 in phosphate-buffered saline (PBS) at pH 7.0. Serum samples were diluted at 1:50 for immunoglobulin A (IgA), 1:200 for IgM, and 1:2,000 for IgG. Triplicate samples were dispensed into microtiter wells for incubation overnight at 2 to 8°C. After incubation, plates were washed twice in PBS containing 0.05% (vol/vol) Tween 20 and incubated for a further 1 h at 37°C with 100 μl of the appropriate peroxidase conjugated anti-human immunoglobulins diluted in PBS containing 0.05% (vol/vol) Tween 20 (1:10,000 for IgG and 1:2,000 for IgM and IgA). After being washed with PBS in 0.05% (vol/vol) Tween 20, the plates were developed by addition of 100 μl of tetramethyl benzidine chromogen for 10 min at room temperature (controlled at 22°C). Reactions were stopped by the addition of 50 μl of 25% (vol/vol) phosphoric acid, and absorbency values were measured at 450 nm with a microtiter plate spectrophotometer. Endpoint titers were calculated as the mean absorbencies against the baseline with coefficients of variations typically of <5% for all triplicate determinations and trend analysis at both dilutions.

#### Determination of antibodies to lipopolysaccharide

LPS was prepared from the *P. aeruginosa* vaccine strain by the method of Westphal and Jann (32). Microtiter plates were coated with 100 μl of 25-μg/ml LPS in 10 mM PBS at pH 7.4 containing 50 mM MgCl2 and incubated overnight at 37°C. Plates were then blocked with 200 μl of 10 mM PBS (pH 7.4) containing 5% (wt/vol) bovine serum albumin and 0.05% (vol/vol) Tween 20 for 15 min at 22°C. Plates were washed twice in PBS containing 0.05% (vol/vol) Tween 80, and 100 μl of pooled serum diluted in PBS (1:200 for IgA and IgM and 1:2,000 for IgG) was added to each well and incubated overnight at 4°C. After being washed in PBS containing 0.05% (vol/vol) Tween 80, the plates were incubated with the appropriate peroxidase conjugated anti-human immunoglobulin (1:2,000 dilution). Tetramethyl benzidine chromogen (1:10,000 dilution) was added to each well, and plates were incubated for a further 60 min at room temperature (22°C). Direct binding and opsonophagocytosis assays

The sera of all subjects were pooled for each of the time points during the study and used to determine opsonophagocytosis of five *P. aeruginosa* strains 385 with a human monocytic cell line (THP-1). THP-1 cells were seeded into 96-well flat-bottom ELISA plates (50 μl) at a density of 1.5 × 10^5 cells per well or diluted to achieve 1.5 × 10^4 cells per well. RPMI-1640 medium containing 10% (vol/vol) fetal calf serum and 10 μg/ml penicillin-streptomycin for 3 days. The cells were then activated to produce a more macrophage-like phenotype by the addition of 50-ng/ml myristyl phorbol ester for 24 h. After activation, nonadherent cells were removed by washing, and the adherent cells were harvested with a cell scraper and suspended in Hanks balanced salt solution (HBSS). Bacteria were opsonized by combining 50 μl of bacteria (equivalent to 10^4/ml) to 20 μl of pooled serum for each of the time points and incubating for 30 min at 25°C. Cells were washed twice in PBS and made to 1 ml, of which 50 μl was added to 500 μl of activated THP-1 cells and again made to 1 ml with HBSS. The cells were incubated for 30 min at 37°C, washed in HBSS containing 250-μg/ml gentamicin sulfate, and further incubated for 10 min to kill adherent nonphagocytosed bacteria. Cells were then washed in PBS, and a 30-μl sample was removed and lysed in 450 μl of water. An additional 10-fold dilution of the sample was made, and 50 μl of this dilution was plated onto nutrient agar to determine the number of CFU. The remaining macrophages with phagocytosed *P. aeruginosa* were incubated for a further 60 min (total time, 90 min) and plated as above to determine the extent of killing of *P. aeruginosa* by the macrophages. Non-serum samples were used as negative controls, and a control sample of pooled serum from cystic fibrosis subjects known to be colonized with *P. aeruginosa* was also included to represent a high-titer sample. P. aeruginosa antigens. The assay was repeated for each time point on five occasions, and a mean value was determined for the results.

#### Statistical analysis

Serum and saliva-specific antibody levels as determined by ELISA absorbance at 450 nm were compared at each time point against day 0 by using a paired two-tailed *t* test. Statistical significance was defined as occurring...
TABLE 1. IgA and IgM total serum immunoglobulin concentrations and specific whole- 
P. aeruginosa cell extract serum antibody levels

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of serum immunoglobulins (g/liter)</th>
<th>No. of whole-cell extract-specific antibodies (absorbance at 450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgA</td>
<td>IgM</td>
</tr>
<tr>
<td>28</td>
<td>0.48</td>
<td>0.28</td>
</tr>
<tr>
<td>0</td>
<td>1.28 ± 0.27</td>
<td>1.72 ± 0.12</td>
</tr>
<tr>
<td>14</td>
<td>1.36 ± 0.28</td>
<td>1.30 ± 0.13*</td>
</tr>
<tr>
<td>28</td>
<td>1.33 ± 0.28</td>
<td>1.22 ± 0.12*</td>
</tr>
<tr>
<td>42</td>
<td>1.30 ± 0.27</td>
<td>1.23 ± 0.12*</td>
</tr>
<tr>
<td>56</td>
<td>1.38 ± 0.28</td>
<td>1.21 ± 0.12*</td>
</tr>
</tbody>
</table>

Values presented are means ± SEM. * value significantly different (P < 0.05)
from Day 0. A total of 24 subjects were observed.

When P values were <0.05. Salivary antibody data were also expressed as a ratio
of salivary albumin levels to adjust for possible salivary flow rate differences
between individual subjects. For the purpose of the immunogenicity study, re-
sponders were defined as those showing at least two or more postdose antibody
titer increases with an increase of at least >15% above predose baseline levels.

Opsonization was extrapolated from colony counts immediately after phago-
cytosis. Bactericidal effect was measured 1 h postphagocytosis and was expressed
as a percentage of difference in the final colony counts.

For the purpose of analyzing the serum IgM-specific antibody response to the
whole-cell extract, one subject was removed as an outlier. For this subject, values
determined on day 42 and 56 were three to six times the group mean. Removal
of this subject from the IgM analysis did not alter the statistical significance
observed for the group mean.

No significant differences were observed for any of the parameters determined
during baseline levels. Hence, of the 24 volunteers who completed the study, 29% were considered IgA responders,
25% were considered IgM responders, and 33% IgG were considered responders against whole-cell P. aeruginosa extracts
(Table 2).

There were no significant changes in the levels of P. aerugi-

nosa-specific IgA salivary antibody levels following immuniza-

tion. Salivary IgG antibodies against whole-cell P. aeruginosa

extract demonstrated an increase from baseline levels at day

14, which waned by day 42 before a more significant second

increase at day 56 (P = 0.027) (data not shown). No statisti-
cally significant changes were observed for either salivary IgA

adverse event reports except for the reported headache, which
occurred 1 day after the initial dose. One serious adverse event
involved a subject who had an epileptic seizure (described as
being of moderate severity) and was hospitalized overnight for
treatment. This subject was subsequently found to have had a
seizure some months previously, which had been not reported
to the investigators, and a family history of epilepsy. No ab-
normal hematological or biochemical test results of clinical
significance were found at screening or at the day 56 follow-up
visit for any subjects. There were no clinically significant
changes in vital signs observed throughout the course of the
study. All patients felt well at the completion of the study.

Total serum immunoglobulins. Total serum immunoglobu-
lins showed no statistically significant changes or trends through-
out the course of the study. Values for IgA and IgM are presented
in Table 1.

Specific antibody responses to whole-cell P. aeruginosa. Se-

rum whole-cell P. aeruginosa extract-specific IgA increased by
day 14 after the first dose, and remained above the day 0 level
for the remainder of the observation period (Table 1). The
group mean showed a statistically significant increase in P.

aeruginosa-specific IgA titers compared to day 0 for day 14
(P = 0.009), day 28 (P = 0.017), and day 42 (P = 0.010) but
failed to reach significance on day 56 (P = 0.053). Whole-cell
P. aeruginosa extract-specific IgM antibody titers also reached
a statistically significant group mean increase from baseline
at day 14 (P = 0.019) (Table 1). No statistically significant
changes were observed for whole-cell P. aeruginosa extract-
specific serum group mean IgG levels (data not shown).

Of the subjects tested, 39 to 65% were positive responders
for all antibody classes and proteins tested. The least number
of responders was to OprI at 39% and 48% for IgA and IgM,
respectively. All other categories showed >50% responders;
however, for the purpose of this analysis, responders were
defined as those showing at least two or more postdose anti-
body titer increases with a least one increase of >15% above
the predose baseline levels. Hence, of the 24 volunteers who
completed the study, 29% were considered IgA responders,
25% were considered IgM responders, and 33% IgG were
considered responders against whole-cell P. aeruginosa extracts
(Table 2).

TABLE 2. Percent responders in each category of
antigen-specific antibody

<table>
<thead>
<tr>
<th>Immunoglobulin class</th>
<th>Whole-cell extract % (n)</th>
<th>OprF % (n)</th>
<th>OprI % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>29 (7)</td>
<td>46 (11)</td>
<td>21 (5)</td>
</tr>
<tr>
<td>IgM</td>
<td>25 (6)</td>
<td>25 (6)</td>
<td>17 (4)</td>
</tr>
<tr>
<td>IgG</td>
<td>33 (8)</td>
<td>4 (1)</td>
<td>33 (8)</td>
</tr>
</tbody>
</table>

Values were determined for any individual showing in serum at least ≥2
postdose antibody titer increases with at least one predose baseline level of
>15%. n, number of subjects who responded of the 24 subjects completing the trial.
or IgG antibodies against any of the *P. aeruginosa* antigens tested when the antibody data were expressed as a ratio of the salivary albumin levels. Salivary IgM-specific antibodies were not measured.

**Outer membrane protein (OprF and OprI)-specific IgA responses.** No significant group mean IgA, IgM, or IgG serum responses were observed for either OprF or OprI. Of the 24 volunteers, 46% were OprF-specific IgA responders, and 33% were OprI-specific IgG responders. To a lesser degree and in decreasing order, the responders were as follows: OprF-specific IgM responders (29%), OprI-specific IgA responders (21%), OprI-specific IgM responders (17%), and OprF-specific IgG responders (4%) (Table 2).

**LPS.** LPS-specific serum IgA titers increased significantly (*P* < 0.05) from baseline levels on day 14, day 28, day 42, and day 56 with maximum increases occurring 28 days post-first dose (Fig. 1). LPS-specific serum IgM titers showed no significant change from baseline on day 14; however, there were statistically significant (*P* < 0.05) decreases in responses for days 28, 42, and 56. LPS-specific serum IgG titers showed a pattern similar to that of LPS-specific serum IgA, compared to day 0, achieving statistically signifi-
significant increases ($P < 0.05$) on all days postdose except day 14 (Fig. 1C).

**Opsonophagocytosis assays.** The number of colonies grown after opsonization and phagocytosis by activated THP-1 monocytes in the presence of pooled serum from Pseudostat-vaccinated individuals is represented in Fig. 2. The results show an increase (34 to 62%) in the number of colonies compared to day 0, with the highest macrophage capture of live *P. aeruginosa* cells in the presence of CF sera, which was used as a control. After a further 60-min incubation period, the percentage of opsonized colonies killed ranged from 45 to 82%, compared to 40% on day 0 (Fig. 3). Killing of bacteria was particularly enhanced on day 14 (82%), at which maximal anti-*P. aeruginosa* IgA responses in serum samples were also observed. After day 14, the levels dropped progressively but were still maintained above baseline values.

**DISCUSSION**

Studies in a rodent model of acute infection have demonstrated that mucosal immunization with a whole-cell killed *P. aeruginosa* vaccine results in enhanced clearance of the bacteria from the lung, as well as reduced mortality (2, 7). In a preliminary study of nine bronchiectasis patients, oral immunization with an enteric coated whole-cell killed *P. aeruginosa* vaccine resulted in the detection of circulating antigen-reactive peripheral blood leukocytes, as well as a significant reduction in the levels of *P. aeruginosa* in the sputum (8). Further evidence to support the development of a whole-cell killed vaccine and an oral immunization strategy for *P. aeruginosa* comes from previous studies of nontypeable *Haemophilus influenzae* (NTHi), where patients with recurrent acute exacerbations of chronic bronchitis were orally immunized with a whole-cell killed NTHi vaccine (4, 5, 6, 20, 28). A recent Cochrane review of six NTHi trials of 440 subjects reported that oral immunization significantly reduced the number and severity of acute exacerbations (13). In those studies, bacterial load was reduced, as determined by the incidence of throat colonization and/or quantitative or semiquantitative bacteriology of sputum samples. In addition, NTHi-specific cellular responses were detected in peripheral blood lymphocytes, following oral immunization with whole-cell killed NTHi vaccine.
This study represents a clinical pilot study designed to demonstrate the immunogenicity and safety of an oral vaccine against *P. aeruginosa* in healthy volunteers. The dose chosen was the lowest dose expected to induce an immune response in humans, based on a scaled-up dose from successful dose-ranging studies in rodents. Future placebo-controlled studies will determine efficacy and optimal dose and treatment regimes with human subjects.

Safety of the vaccine in healthy individuals was confirmed in the absence of any probable or possible adverse events, and no hematological or biochemical profile derangements were recorded for any subjects throughout the study. While 20 adverse events were recorded, these were not considered of clinical significance (the exception being one case of epilepsy missed on initial screening), and none were attributable to the study vaccine being administered. Upper respiratory tract infection represented 60% of the adverse events reported with twice the number of upper respiratory tract infections following the second dose compared to the first dose. However, there was no temporal relationship to either the day of dosing or in the time of occurrence.

The vaccine was demonstrated to be immunogenic. Of particular note is the significant increase in IgA-specific antibody responses against both whole-cell extract and LPS in the post-immunization observation period. Confidence in the consistency and specificity of these changes was further supported by comparison with group mean total serum IgA, which did not follow a similar trend. A sustained-specific IgG LPS antibody response was also observed postimmunization. The opsonization results clearly showed a substantial increase in ability of pooled sera from the study volunteers to promote phagocytosis of *P. aeruginosa* by a human macrophage cell line. The results confirm the specificity of the response observed and that antibodies induced were opsonizing for *P. aeruginosa*. Opsonizing antibody responses have been shown to be important for protection against *P. aeruginosa* infection in CF patients (24, 30). Hostoff et al. (15) reported on the importance of IgA receptors (Fc alpha R) on mucosal phagocytes and suggested an important role in defense of mucosal surfaces. They demonstrated high levels of expression of Fc alpha R on neutrophils obtained from bronchoalveolar lavage fluid of cystic fibrosis patients and showed that neutrophil superoxide production was enhanced by IgA. Significant IgA responses following pneumococcal polysaccharide vaccination showed that the IgA induced a comparable receptor-mediated phagocytosis response, as did IgG, and that the IgA holds an important leukocyte receptor which provides immunity against *Streptococcus pneumoniae* (25). In this study, both opsonization and killing peaked at day 14 post-first immunization; although there was a decline in functional activity after this observation point, functional activity remained greater than the preimmunization levels. The two booster immunizations did not enhance either opsonization or killing. Further studies are required to investigate the kinetics of the functional antibody data, particularly in response to booster immunization.

The overall salivary immune responses did not reach statistical significance and were variable at each time point of the study. These observations probably reflect the inherent difficulties of measuring antibody responses in mucosal secretions, particularly following oral immunization. Despite the absence of a detectable and consistent salivary antibody response to the vaccine, this does not preclude the possibility of a mucosal immune response in the lungs or on other mucosal surfaces. If memory T cells and B cells are migrating to other mucosal surfaces from the gut, a measurable response may not be observed until booster encounter with antigen at these surfaces. IgA can reach external secretions by passive paracellular diffusion and may have a role in local defense on mucosal surfaces in addition to locally produced IgA antibodies. Furthermore, leakage of serum IgA onto mucosal surfaces is suspected to increase in inflammatory situations such as those that exist in the CF lung (22). In the context of the results of this study, Berstad et al. (1) noted an absence of salivary antibody response to a whole-cell pertussis vaccine administered nasally to human volunteers, despite strong serum antibody responses for IgA and IgG. Recently, a hybrid OprF-OprI *P. aeruginosa* vaccine was tested with human volunteers by intranasal application, which resulted in serum IgA and IgG responses in 75% of the volunteers (19). Although saliva and nasal washings were taken for analysis in that study, the results were not reported, implying that no responses may have been detected. Studies of oral whole-cell vaccination with an animal model have shown that protection against *P. aeruginosa* challenge can be observed in the absence of any significant serum or bronchoalveolar lavage specimen antibody response (7). These findings are also consistent with results observed for whole-cell killed NTHi oral immunization of subjects with chronic bronchitis, which did not stimulate a specific antibody response in saliva, although the vaccine was highly efficacious (6). Dose-ranging studies of mice (M. Dunkley, unpublished data) have shown that enhanced clearance at the lowest dose was achieved in the absence of any detectable serum antibody response. Increasing the dose by 1 log, which is equivalent to the single doses given in this study (2.6 mg/kg versus 2.1 mg/kg), resulted in better clearance in the mouse and the detection of statistically significant increases in serum IgA and IgM, as was indeed observed in this trial. These results may suggest an important role for serum IgA in the protection against *P. aeruginosa* lung infections.

Studies of this vaccine with an animal model have indicated that both antibody and T cells are important in protecting rodents from an acute lethal challenge of *P. aeruginosa* to the lungs (2, 7, 10, 11). However, some evidence points to a detrimental effect, at least in chronically colonized CF patients where increasing serum IgG antibody levels to *P. aeruginosa* LPS and exotoxin A and the resulting immune complexes formed have been associated with a poor prognosis (21). Kronborg et al. (17) showed that in sputum, immune complexes were almost entirely composed of LPS-specific IgG. Studies have shown that the sera of colonized patients have high titers of poorly opsonizing antibodies (26) and that the opsonizing ability of sera decreases upon conversion to the mucoid form and the onset of chronic colonization (29, 30). Of importance, the results from this study clearly demonstrate that the antibody response induced was opsonogenic. The specificity of opsonizing antibodies and their protective role requires further study (12, 23, 24).

In a recent review (27), we concluded that mucosal immunization was a potential viable option for protection against *P. aeruginosa* infections in at-risk patients. This study has demonstrated that oral immunization against *P. aeruginosa* is safe and induces a significant serum antibody response, notably of the IgA isotype. In addition, an increase in functional opsonization and killing by
macrophages was demonstrated in vitro with postvaccination serum. Proof of concept with human subjects, based on the extensive preclinical studies with animal models, has been established. Further studies will involve dose-ranging studies and characterization of the immune response induced with respect to serotype specificity, cell-mediated immune mechanisms, the identification of the functional opsonins and their antigenic specificity, and the kinetics of the response.

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