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Immune responses in the airways by nasal vaccination with systemic boosting against *Pseudomonas aeruginosa* in chronic lung disease☆

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ABSTRACT

Rationale: Pneumonia caused by *Pseudomonas* (*P.*) *aeruginosa* is a leading cause of morbidity and mortality in patients with chronic lung diseases. Systemic vaccination in patients with cystic fibrosis has been only successful in part. Mucosal vaccination could lead to enhanced airway immunogenicity. Pathogen specific secretory IgA antibodies could prevent bacterial invasion into the lung mucosa.
Objectives: A phase 1–2 mucosal vaccination trial with an intranasal *P. aeruginosa* vaccine was performed.
Methods: 12 patients with chronic lung diseases (8 COPD, 2 cystic fibrosis, 1 bronchiectasis, 1 histiocytosis X) were vaccinated three times intranasally followed by a systemic booster vaccination with a recombinant hybrid protein encompassing the main protective epitopes of two outer membrane proteins of *P. aeruginosa*. Mucosal and systemic antibody responses were measured after boosting and after a half-year follow-up compared to a representative control cohort.
Measurements: Specific IgG and IgA antibodies in the patient's sera, saliva and sputum were determined by enzyme-linked immunosorbent assay (ELISA) and IgG subclass distributions were defined with monoclonal mouse antibodies.
Results: Both forms of vaccination were well tolerated. Significant elevated IgA and IgG antibodies could be measured in sputum, saliva and in the sera of 11/12 patients.
Conclusions: Mucosal vaccination followed by systemic boost with an outer membrane protein vaccine against *P. aeruginosa* leads to airway immunogenicity against the pathogen. Further clinical trials should elucidate the protective efficacy of this vaccination method.
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1. Introduction

Pseudomonas (*P.*) *aeruginosa* is one of the most relevant pathogens causing lung infections in patients with chronic diseases such as cystic fibrosis (CF), bronchiectasis and chronic obstructive pulmonary disease (COPD) [1–3]. Therapy of *P. aeruginosa* infection is often complicated by antibiotic resistances, for review see Ref. [4]. Hypermutable *P. aeruginosa* strains responsible for the development of antibiotic resistance have been found in high frequency in the lungs of CF patients and also recently in the lungs of COPD patients [5]. Vaccination of patients with chronic lung disease could therefore improve their clinical prognosis. However, prevention of infection by systemic vaccination in CF patients has been only partly effective [6]. A recent phase III clinical trial demonstrated significant, but limited protection against vaccine type strains of *P. aeruginosa* by a systemic vaccine based on flagella protein [7]. As yet, no phase III clinical vaccination trials against *P. aeruginosa* infection in COPD patients have been published. For the efficacy of vaccination against mucosal pathogens the route of vaccination plays an important role. While systemic vaccination predominantly induces a systemic immunity, nasal and oral application leads to a specific homing into the lamina propria at local and distant mucosal tissues. Mucosal vaccines may thus offer an enhanced protection of the airway mucosa by inducing pathogen specific mucosal secretory IgA antibodies preventing bacterial invasion into mucosal tissues [8–11] and neutralizing the invading pathogen at the entry site of the body.

In the previous years we have developed a *P. aeruginosa* vaccine based on highly conserved outer membrane epitopes of the pathogen [12]. A recombinant fusion protein of the immunogenic determinants of OprF and OprI, Met-Ala-$(His)_6$OprF190-342-

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OprI21-83 (OprF–OprI), provided synergistic immunogenicity and protection in animal models. It was successfully tested as a systemic vaccine adsorbed on $Al(OH)_3$ for safety and immunogenicity in clinical trials [13,14]. In addition to the systemic formulation, a mucosal formulation was successfully tested in animals and also in healthy volunteers [15,16]. The mucosal formulation contained the OprF–OprI protein mixed with sodium dodecylsulfate into a nasal gel. A nasal vaccination with a systemic booster vaccination schedule compared to nasal vaccination alone showed a trend to higher levels for the IgG isotype in the systemic booster group. Moreover, nasal vaccination irrespective of the booster schedule lead to a significant increase of *P. aeruginosa* specific IgG- and IgA-antibody response in the pulmonary airways of healthy volunteers [16,17]. In a recent study by Wu et al. OprF has been demonstrated to be the major binding site for interferon gamma (IFN-γ) in *P. aeruginosa* [18]. IFN-γ induces quorum sensing signals in *P. aeruginosa* that lead to the expression of toxins such as PA-1 lectin, facilitating the invasion of the pathogen in the host tissue. OprF, therefore, may play an important role in the adaptation of *P. aeruginosa* to the host immune defense, suggesting a potential new effector mechanism for OprF containing vaccines.

In contrast to conventional systemic vaccines, nasal vaccines are particularly prone to enzymatic degradation and mechanical clearance. Inflammation and enhanced mucus secretion may additionally compromise the immunogenicity of a nasal formulation. Our OprF–OprI vaccine was as yet successfully tested only in healthy volunteers. The present study aimed to assess the tolerability and immunogenicity of the nasal OprF–OprI vaccine in patients with chronic pulmonary conditions who are at risk of fatal *P. aeruginosa* infection.

2. Materials and methods

2.1. Vaccines

The vaccination hybrid protein OprF–OprI consisting of the mature outer membrane protein I (OprI) and amino acids 190–342 of OprF of *P. aeruginosa* was expressed in *E. coli* and purified by Ni^{2+} chelate-affinity chromatography as described previously [13,14,19].

For the systemic vaccine OprF–OprI was adsorbed to $Al(OH)_3$ as described previously. One dose of the vaccine contained 100 μg of OprF–OprI, 0.3 mg of $Al(OH)_3$ (Superfos, Vedbaeck, Denmark) and 0.05 mg of thimererosal (Caesar and Lorenz, Hilden, Germany) [13]. The nasal vaccine was produced as described previously. Briefly, an aqueous solution of the OprF–OprI protein was emulgated into a gel containing 1% OprF–OprI, 45% sodium dodecylsulfate (Merck, Darmstadt, Germany) and 5% aerosil (Caesar und Lorenz, Hilden, Germany) [15,16]. The gel was stored in 1 ml aliquots at 4 °C. One dose of the vaccine (100 μl) contained 1 mg of OprF–OprI protein.

2.2. Patients and vaccinations

12 outpatients from the Department of Pneumology of the Freiburg University Hospital (6 male; 6 female, Table 1) between 22 and 70 (mean 56.4) years of age gave their written informed consent in accordance with institutional review board-approved protocols. Local ethic committee approval has been given. As specified by the German regulations for vaccination studies, the study protocol and protocols concerning the preparation of the vaccines and the laboratory and animal safety testing of the vaccines were deposited at the Paul Ehrlich Institute (Langen, Germany). All patients underwent a physical examination and their medical histories were taken to rule out any conditions which would lead to exclusion from the study. Current *P. aeruginosa* infection at the beginning of the study has been ruled out for all patients but previous exposition cannot be excluded for patients 5 and 6 since IgG levels were higher than in the other patients. In addition, a representative out-patient control cohort with age-matched patients suffering from equivalent diseases has been recruited in order to receive comparable results of *P. aeruginosa* out-patients prevalence in the observed society (Table 2).

Table 1
Sex, age, BMI and the disease for each patient.

Patient	Age [years]	Sex	BMI [kg/m^2]	Disease
P1	65	Male	28.5	COPD
P2	60	Female	35	COPD
P3	22	Female	13.4	CF
P4	65	Female	28.8	Bronchiectasis
P5	68	Female	23.6	COPD
P6	64	Female	37.8	COPD
P7	68	Male	21.3	COPD
P8	58	Male	21.9	Histiocytosis X
P9	37	Male	22.4	CF
P10	54	Female	27.1	COPD
P11	54	Male	21.6	COPD
P12	70	Male	22.7	COPD

COPD: chronic obstructive pulmonary disease, CF: cystic fibrosis, and BMI: body mass index.

The patients received three single intranasal immunizations of 100 μl OprF–OprI gel. The second nasal immunization was administered on day 15 (±9 days) after primary vaccination, the third immunization on day 29 (±8 days). Before application the gel was warmed up to room temperature. Instillation was made with a 25 gauge plastic catheter (Abbocath, Abbott, Wiesbaden, Germany) into either the right or left nasal cavity above the middle concha nasalis. Followed by a systemic booster vaccination two to four weeks after the last nasal application with 100 μg of OprF–OprI adsorbed to $Al(OH)_3$ injected into the deltoid muscle of the left or right arm.

2.3. Sampling procedures and processing

For the determination of OprF- and OprI-specific antibodies venous blood samples were taken prior to immunization (pre), between week three and six (post) and 6 months after the systemic booster vaccination. Saliva and sputum samples were also taken three to six weeks and 6 months after the systemic booster vaccination.

Induced sputum (IS) was obtained by inhalation of nebulized (Pariboy, Pari, Starnberg, Germany) hypertonic saline (5.85% sodium chloride, Braun, Melsungen, Germany) for 2 min. After the inhalation period, the patients were asked to cleanse their mouth with water and dry it in order to minimize contamination with saliva. Expectorated sputum was subsequently collected over two minutes. The inhalation and expectoration procedure was repeated five times in order to augment the amount of recovered sputum. To avoid bronchial obstruction the patients inhaled 200 μg Salbutamol with a metered dose inhaler (Epaq, 3M Medica, Neuss, Germany) prior to the first saline inhalation. Sputum and saliva samples were mixed immediately with 2 μl of 0.5 M ethylenediaminetetraacetic acid (EDTA) (Dako, Hamburg, Germany) and 1 μl of 100 mM phenylmethylsulfonylfluoride (PMSF) (Dako, Hamburg, Germany) which was added to 100 μl IS for inhibition of proteases and metalloproteases, respectively. Saliva and IS samples were stored at −70 °C, sera in aliquots at −20 °C. IS was centrifuged immediately after thawing at 4 °C with 15,000 × g for 25 min and the supernatant was used for further analysis.

OprF–OprI-specific antibodies were assessed by ELISA as described previously [20,16]. Briefly, 96-well microtiter plates were coated with OprF–OprI at 1 μg/ml, blocked by a 0.2% bovine serum albumin (BSA, Sigma, Munich, Germany) solution, incu-

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