

RECOMBINANT OprF-OprI AS A VACCINE AGAINST *PSEUDOMONAS AERUGINOSA* INFECTIONS*

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SUMMARY

A vaccine against *Pseudomonas aeruginosa* based on recombinant outer membranes has been developed. After intramuscularly injection into patients with severe burns, antibodies against *P. aeruginosa* were induced. Vaccination was well tolerated. Intranasal application of the vaccine into volunteers induced specific sIgA antibodies. We conclude that the newly developed vaccine may be suitable for protection of the main risk groups of *P. aeruginosa* infections. In particular for the protection of burn patients and patients with cystic fibrosis.

INTRODUCTION

Pseudomonas aeruginosa represents a leading cause of nosocomial infections and pneumonia in hospitals (Gallagher and Watanakunakorn, 1989; Gordon et al., 1998; Holder, 1988; Holzheimer et al., 1990; Pennington, 1994) pathogen affects mainly immunocompromised patients, such as patients with large burns (McManus et al., 1985; Pruitt et al., 1984, 1998) or patients under immunosuppressive or cytostatic therapy for the prevention of organ rejection after transplantation (Korvick et al., 1991) or for cancer treatment (Griffith et al., 1989). Also compromised local defence mechanisms, such as an impaired mucociliary clearance in patients with cystic fibrosis (Burns et al., 2001) artificial ventilation or paraplegia can enhance the susceptibility to pulmonary *P. aeruginosa* infections. The eradication of

Pseudomonas frequently proves difficult due to antibiotic resistance and the ability to form a biofilm in case of chronic infection (Hanberger et al., 1997; Hancock, 1986; Hoiby et al., 2001; Hsueh et al., 1998; Srikumar et al., 1988; Tassios et al., 1998).

Clearance of *P. aeruginosa* in systemic infection is mediated predominantly by antibodies of the IgG1 isotype and by complement-dependent opsonisation (Hong and Ghebrehiwet, 1992). Secretory IgA antibodies are likely to be the first line of defence (McGhee et al., 1999) for prevention of adherence and subsequent infection of mucosal tissues like the lung, the urogenital tract or the paranasal sinuses. A clinical vaccine against *P. aeruginosa*, therefore, should induce protective antibodies of both isotypes.

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Since the 1960s a number of experimental vaccines were developed and tested for the prevention of *P. aeruginosa* infections in burn patients. The most promising of the vaccines tested were the two vaccines based on lipopolysaccharides (LPS) as antigens (PseudogenTM, a heptavalent preparation, and PEV-01 a 16-valent vaccine). Both vaccines appeared to effectively stimulate the induction of antibodies against all O-serotype strains contained in the vaccine when tested in burn patients and lead to a reduction of mortality from *P. aeruginosa* infection in this group of patients (Alexander and Fisher, 1974; Jones et al., 1979; Miller et al., 1977). Clinical studies with PseudogenTM vaccine were also carried out in patients with cystic fibrosis (CF), malignancies, and in intensive care patients with respiratory failure. However, both LPS vaccines did not meet the approval for routine clinical use because of their toxicity associated with their lipid A fraction. Subsequently, subunit vaccines based on purified oligosaccharides from LPS and conjugated to *P. aeruginosa* exotoxin (Cryz et al., 1987a,b,1988) or mucoid exopolysaccharide [alginate] (Pier, 1982,1985,1994,1997) or isolated flagella were shown to be less toxic and have been successfully shown to elicit antibodies in a number of volunteers and patient groups (Cryz et al., 1987a,b,1988; Pier, 1982,1985,1994, 1997). However, currently there is still no approved vaccine against *P. aeruginosa* available for routine use for which safety and efficacy have been shown in clinical trials in patients from one of the major risk groups of *P. aeruginosa* infection.

Our research over the last decade has focused on the development of a vaccine against *P. aeruginosa* based on the outer membrane proteins [OPRs] of *P. aeruginosa*. A vaccine based on OPRs may have several advantages. First, they

are highly conserved and induce a cross protective immunity among all 17 known *P. aeruginosa* serotypes (Mutharia et al., 1982; von Specht et al., 1987). Second, OPRs can be produced by recombinant DNA technology free of contaminating LPS. Third, the cloned genes of OPRs would be applicable for naked DNA immunisation (Cohen, 1993; Donnely et al., 1994; Price et al., 2002) or could be transfected into special vectors like non-pathogenic *Salmonella* strains to induce a mucosal immune response (Kraehenbuhl and Neutra, 1992; Toth et al., 1994). The efficacy of OPRs as a vaccine candidate was shown by us and other research groups (Finke et al., 1990, 1991; Gilleland et al., 1984,1988; Matthews-Greer and Gilleland, 1987; von Specht et al., 1987,1995) in various animal models. We have cloned the major OPRs, outer membrane protein F (OprF) (Duchêne et al., 1988) and outer membrane protein I (OprI) (Duchêne et al., 1989). After identification of the protective epitopes on both proteins we have generated a recombinant hybrid protein consisting of the entire OprI molecule fused to the carboxy terminal sequence (aa 190-342) of OprF (von Specht et al., 1995). The hybrid protein comprised the presence of the main known protective epitopes (Gabelsberger et al., 1997; Gilleland et al., 1995; Gilleland and Gilleland, 1995; Hancock and Wong, 1997). A modified histidine tagged hybrid protein Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃, resulted in a synergistically enhancement of protection in an immune suppressed mouse model (von Specht et al., 2000). Subsequently two vaccine formulations for different clinical applications were developed. A systemic vaccine formulation aimed for protection by means of IgG isotype antibodies against systemic infections like in burn patients. It consists of Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃ adsorbed to Al(OH)₃. The second for-

mulation was designed for protection by the induction of s-IgA antibodies against mucosal infections, like isolated lung infections in Cystic Fibrosis patients. The mucosal vaccine is based on a nasally applicable gel, produced by mixing the Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-

OprI₂₁₋₈₃ protein with sodium dodecyl-sulfate (SDS) and aerosil. In this article, we summarise the data we recently generated with both vaccine types in phase 1 and phase 2 clinical trials in volunteers and selected patient groups.

METHODS

Expression of Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃ protein in *E. coli* and purification

The expression and purification of Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃ protein has been described in detail (Mansouri et al., 1999).

Vector

The recombinant vector pTrc-His-F-I, carrying the hybrid gene encoding parts of OprF and OprI from *P. aeruginosa*, was constructed as described previously (Gabelsberger et al., 1997). The vector was transfected into *E. coli* XL-1 Blue bacteria and the expression of Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃ protein induced using standard procedures (Ausubel et al., 1997).

Purification

Forty grams cell wet mass was lysed by one passage through a Gaulin press at 1,200 psi. The cell extract was clarified at 48,000 x g for 90 min at 4°C and passed through a 0.45 µm filter. The crude extract was purified by affinity chromatography on a Ni-NTA super-flow column. The specific eluate was concentrated by centrifugation in MACROSEP 10 units by a factor of 3. The pH of this eluate was lowered to 5.9 by adding 0.02 mol/L NaH₂PO₄ monohydrate, pH 3.0, incubated at 4°C overnight and then clarified for 10 min at 4°C and 5000 x g to precipitate the lipopolysaccharides. The pH was re-titrated to 7.0-7.2 by adding a 0.1 N

NaOH solution drop wise. The neutralised protein solution was filter (0.22 µm) sterilised and stored at 4°C overnight. Finally, the purified protein was concentrated to about 1 mg/ml by ultra filtration using a stirred Amicon cell and a YM10 membrane and then extensively dialysed against sterile, pyrogen-free PBS at 6°C for 20 h.

Vaccine preparation 1 (Parenteral vaccine) (Mansouri et al., 1999)

Recombinant OprF-OprI was adsorbed to Al(OH)₃, (Alhydrogel™), Superfos, Vedbaek, Denmark and Thimerosal (Caesar & Lorenz, Hilden, Germany) was added as a preservative. A Thimerosal stock solution was prepared, using a sterile, pyrogen-free physiological saline solution. For the 1 mg/ml vaccine preparation, a dispersion of 3% [w/v] of Al(OH)₃ was mixed with the OprF-OprI solution and the Thimerosal stock solution to yield final concentrations of OprF-OprI: 1 mg/ml, Al(OH)₃: 3 mg/ml and Thimerosal: 0.05 mg/ml. Al(OH)₃ and the OprF-OprI solution were mixed and stirred for 30 min, and the Thimerosal solution was then added. This was followed by additional stirring for 10 min. For the 0.1 mg/ml OprF-OprI vaccine preparation, pyrogen-free physiological saline solution was added to yield final concentrations of 0.1 mg/ml OprF-OprI, 0.3 mg/ml Al(OH)₃ and 0.05 mg/ml Thimerosal. Aliquots of one ml were aseptically introduced into sterile pyrogen-

free glass vials, and the vials stoppered and sealed.

Vaccine Preparation 2 (*Mansouri et al., 1997*)

80 mg Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃ protein were mixed with 0.54 g sodiumdodecylsulfate and 0.6 g aerosil. The emugel was stirred three times for one minute at 300 rpm in an UMC 5-stirring machine. Aliquots were aseptically introduced into sterile pyrogen-free cryovials and stored at 4°C.

Safety evaluations

The identity and purity of the Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI₂₁₋₈₃ protein and the expression of the relevant epitopes was assessed by western blot

analysis and epitope specific monoclonal antibodies as described in detail recently. After intramuscularly injection of vaccine preparation 1 and after intranasal application of vaccine 2 into rats no signs of histopathological changes were detectable (*Mansouri et al., 1997,1999*).

All volunteers and patients gave their informed written consent in accordance with institutional review board-approved protocols. As specified by the German regulations for vaccine studies, protocols concerning the preparation of the vaccine and the laboratory and animal safety testing of the vaccine were deposited at the Paul Ehrlich Institute, Langen, Germany.

VACCINATION STUDY 1: DOSE FINDING AND SAFETY STUDY IN HUMAN VOLUNTEERS (*Mansouri et al., 1999*)

Subjects and study plan

Thirty-two healthy volunteers (16 male; 16 female; >18 years of age) were randomly allotted to 4 groups. All volunteers received three consecutive injections of the vaccine into the deltoid muscle of the left arm with 20 µg (0.2 ml of 100 µg/ml), 50 µg (0.5 ml of 100 µg/ml), 100 µg (0.1 ml of 1 mg/ml) or 500 µg (0.5 ml of 1 mg/ml) OprF-OprI, respectively, at 4-week intervals and a fourth injection after six months at the same dose. All volunteers underwent a physical examination and had histories taken to rule out any conditions which would have necessitated exclusion from the study. Before, two and 14 days after each vaccination, blood samples were taken and sent to the clinical laboratory for a complete blood count and evaluation of the liver specific enzymes, creatinine and urea. Reactions to the vaccine were assessed for 3 consecutive days and documented by the volunteers. The local and systemic responses were

graded on a subjective scale of 0 to 3, with the respective scores representing absent, mild, moderate and severe reactions. The vaccinees were instructed to take their temperature before and 12, 24, 48 and 72 hours after vaccination. In addition, each volunteer underwent a physical examination two days after vaccination. For the determination of OprF- and OprI-specific antibodies, venous blood samples were taken on day 0 (prior to immunisation), and two weeks after each vaccination.

Analysis of the immune response

Before and two weeks after each vaccination antibody titres against OprI, OprF and OprF-OprI were determined by ELISA. A significant increase in antibody titres within all the different dosage groups could be measured. The specificity of the antibodies against native *P. aeruginosa* OprF and OprI was confirmed by Western blotting (data not shown). Wild type OprI (6kD) and

Table 1: IgG antibody titres against OprF-OprI in volunteers vaccinated with 100 µg vaccine dose data are summarised from *Mansouri et al., 1999*

Day	Vaccinations given	IgG antibody titre [mean (SD)]
0	0	200
14	1	1218 (623)
42	2	1503 (448)
70	3	3645 (505)

OprF (33kD) were both recognised by the immune sera. Considerable differences were observed between the dosage groups and also between volunteers belonging to the same dosage group.

Statistical analysis showed that after only one vaccination a maximal response was observed for the groups which had received the 100 µg (table 1) or the 500 µg dose. No statistically significant increase of specific antibody titer was measured in these groups after the first and second revaccination. After vaccination with the 20µg OprF-OprI dose, a significant antibody response was measured only after revaccination. Six months after the third vaccination the antibody titres against OprF-OprI were still significantly elevated in all groups. A further booster vaccination after 6 month induced a 3-10 fold increase of the specific antibody titres (Table 1).

Systemic protection against *P. aeruginosa* is mediated in humans predominantly by specific IgG1 antibody and both antibody-mediated and comple-

ment-mediated phagocytosis (*Hong and Ghebrehiwet, 1992*). To address the question whether the vaccine would be protective in patients, IgG subclasses of antibodies against OprF-OprI were determined. In all groups a significant increase in IgG1 antibodies was observed. In addition binding of serum C1q on *P. aeruginosa* coated plates was tested by ELISA (*Eckhardt et al., 2003*) before immunisation and after the third vaccination. A significant increase of C1q binding to antibodies was detected after the third vaccination in all 26 sera tested.

The ability of the OprF-OprI vaccine to boost the opsono-phagocytic efficacy of the sera of the volunteers was measured by incubation of viable *P. aeruginosa* bacteria (ATCC strain 27313) with the sera of the volunteers before and after the third vaccination. The OprF-OprI hybrid protein vaccine demonstrated the ability to boost the opsono-phagocytic activity of the antisera obtained from 73% of the volunteers tested by this assay (*Mansouri et al., 1999*).

CLINICAL TRIAL IN BURN PATIENTS (*Larbig et al., 2001*)

Study population

Eight adult burn patients with the following inclusion/exclusion criteria:

Inclusion criteria: Age between 18-60 years, II° or III° burn, burns covering between 35% and 55% of the total body surface, ABSI score (*Tobiasen et al.,*

1982) between 6 and 10.

Exclusion criteria: Patients with any concomitant diseases, patients with electrical burns, adults whose case records include a former confirmed infection with *P. aeruginosa*, suspected or documented hypersensitivity against one

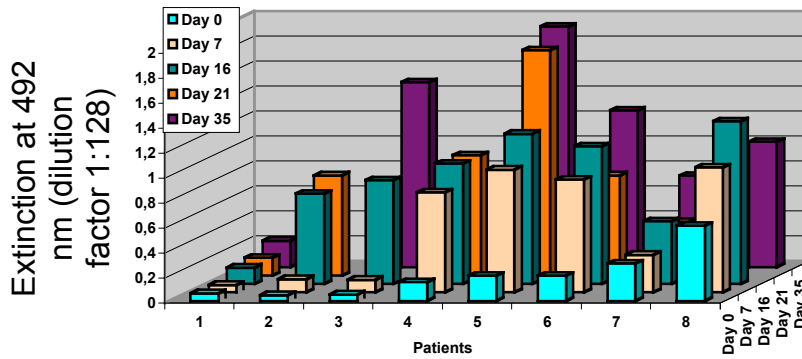


Figure 1: Anti OprF-OprI specific antibody in sera of burn patients detected by ELISA. Patients were vaccinated intramuscularly with 100 μg OprF-I three times at days 0, 7 and 21.

of the substances used in the vaccine or any chemically related substances or pregnancy.

Dose and administration

OprF-OprI was given as an intramuscularly injection into the left upper arm, if not possible due to a burn injury the right side was taken. If both arms were burned we applied the vaccines into the gluteal muscle. As far as possible we stuck to the administration schedule: 1 ml OprF-I (=100 μg) into the left arm, 0,5 ml Tetanol (Tetanus-Toxoid, at least 40 I.E per ml, Chiron Behring, Germany) injection into the right arm, 1 ml Tetagam injection (100-170 mg Immunglobulin, Chiron Behring, Germany) into the left gluteal muscle. The 100 μg dose was chosen because it has been demonstrated to be effective and adequate to induce sufficient antibodies against OprF-I in our volunteer study (Mansouri et al., 1999).

Local and systemic responses were graded with a scale from 0 to 3, with scores representing absent, mild, moder-

ate, and severe reactions, respectively. Body temperature, blood pressure and heart rate were measured before vaccination and 1, 2, 4 and 24 h after each vaccination. In addition, each patient underwent a physical examination 2 days after vaccination.

Response to vaccination in burn patients

Antibody titres against OprF-OprI and tetanus were determined by ELISA before each vaccination and at days 7, 16, 21 and 35. Eleven patients were enrolled and received at least 2 vaccinations. The patients were 21 to 60 years of age (2 females and 9 males). The mean age of all patients was 39 years. Three out of the 11 patients died during the study time because of cardiovascular complications. Eight patients received the three scheduled doses of the vaccine and completed all the post vaccination follow-up visits, and the data on these patients are presented in this study. The burned skin areas covered between 35 and 48% (mean 38%) of the body sur-

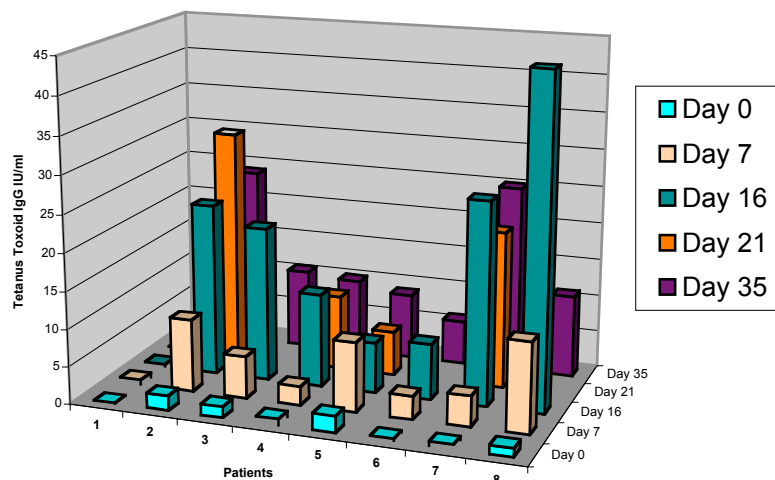


Figure 2: Antibody response against Tetanus toxoid. Patients were vaccinated at the day of admission to the hospital. Antibody response was measured at days 7, 16, 21 and 35.

face. The ABSI score was between 7 and 10 (mean 8). Those patients, who died during the study suffered from the largest burn surfaces (46% or above) and had the highest ABSI scores (9 or higher). The serological tests (ELISA) for detection of antibodies against *P. aeruginosa* and tetanus toxoid showed for 7 patients seroconversion (= at least 3 times higher titre than the pre-vaccination value). The kinetics of the serum

antibody against OprF-I and the tetanus toxoid responses are given in Figures 1 and 2.

The vaccine was well tolerated. No serious side effects were observed. The vaccination did not appear to enhance an inflammatory response in the burn patients. None of the subjects acquired systemic *P. aeruginosa* infections during or after the treatment of their burns.

MUCOSAL VACCINATION TRIAL IN VOLUNTEERS (Göcke et al., 2003)

Study subjects and study plan

Twelve healthy male volunteers (mean age 24.3, range 21.8 to 26.7 years) were included in this study. Exclusion criteria were, beside current or chronic conditions, a previous *P. aeruginosa* infection. Serum and saliva were assayed for total IgG and IgA to exclude undiscovered humoral immune defects. 100 μ l of the emulgel, containing 1 mg OprF-I were applied to the concha

nasalis for the mucosal vaccination, while the systemic vaccination was performed by injection of 1 ml, containing 100 μ g OprF-I, into the deltoid muscle.

We compared two vaccination schedules, one with three consecutive nasal vaccinations at three weeks intervals, the other a combined mucosal/systemic schedule with two nasal vaccinations followed by a systemic booster, also at

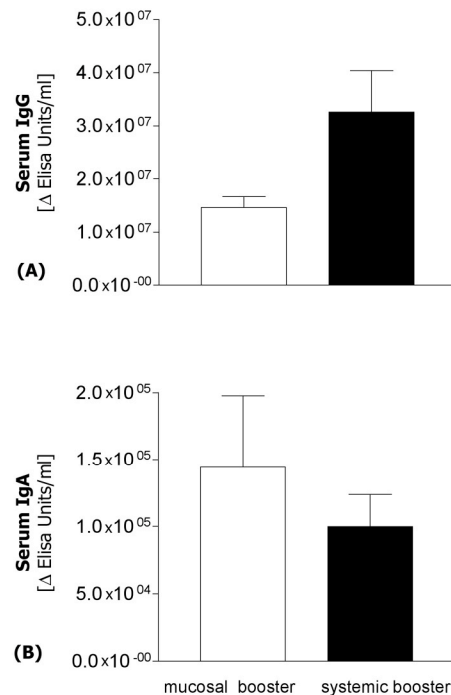


Figure 3: Increase in serum antibody levels against OprF-I of *P. aeruginosa* of IgG and IgA isotype (A and B, respectively) after 3 consecutive nasal ("mucosal booster") or two consecutive nasal vaccinations followed by a systemic booster ("systemic booster"). For further details of the study see "Mucosal vaccination trial in volunteers" in the text and reference (Göcke et al., 2003).

three weeks intervals. The participants were randomly assigned to the two schedules (n=6 per group).

The volunteers were monitored for adverse effects for 5 days after each vaccination by physical examination, blood samples, and body temperature. The induction of OprF-I-specific antibodies was analysed by comparing blood and saliva samples obtained prior to the primary and 4 weeks after the second booster vaccination. OprF-I-specific antibodies were determined as described previously (Mansouri et al., 1999).

Results of the mucosal vaccination study

Apart from a brief local discomfort (burning, tickling in the nose and tension in the muscle) and an occasional episode of fever of less than 24 hrs (n=3 in 36 vaccinations), no adverse effects were observed. All vaccinees showed a seroconversion irrespective of the vaccination schedule. The systemic booster elicited OprF-I-specific IgG antibody titres in serum twice as high as the nasal booster, while specific IgA antibodies did not differ between both vaccination groups (Figure 3). The different booster schedules appeared not to affect the levels of mucosal antibodies as obtained in saliva (Göcke et al., 2003).

DISCUSSION

As already pointed out in the introduction, *P. aeruginosa* is a leading cause of morbidity and mortality in immunocompromised patients. The pathogen can cause severe and often fatal sepsis in burn patients, cancer patients receiving chemotherapy or transplant patients treated with immunosuppressive drugs. Beside causing septicaemia *P. aeruginosa* infections occur frequently in organs suffering from a local impairment of immune barriers. *P. aeruginosa* is the main cause of nosocomial pneumonia in the United States (Pennington, 1994). Chronic lung infection by mucoid strains of *P. aeruginosa* is the leading cause of death in CF-patients (Koch and Hoiby, 1993). Other risk groups are patients with artificial ventilation or paraplegia for lung infection and carriers of contact lenses for eye infection with *P. aeruginosa*.

Systemic vaccination with LPS based vaccines against *P. aeruginosa* was shown in the 1960s to reduce the incidence of *P. aeruginosa* sepsis and to reduce the mortality from this organism (Alexander and Fisher, 1974; Jones et al., 1979; Miller et al., 1977). However, due to endotoxic complications these vaccines were not approved for routine clinical use. We developed a vaccine based on recombinant outer membrane proteins. The protective efficacy of native outer membrane proteins of *P. aeruginosa* against *P. aeruginosa* infection has been shown in animal models by us and various other research groups, and recently in burn patients by researchers of the Cheil Jedang Corp. (Korea) (Finke et al., 1991,1990; Gilleland et al., 1984,1988; Matthews-Greer and Gilleland, 1987; Jung et al., 2000; Kim et al., 2000). However trace contaminations with *P. aeruginosa* LPS in this native outer membrane preparations induced LPS specific antibodies and

resulted in systemic and local side effects by the vaccination (Kim et al., 2000).

We therefore choose the recombinant approach. A hybrid protein carrying the known protective epitopes of the main outer membrane proteins F and I was expressed in *E. coli*. Systemic vaccination of volunteers and burn patients was well tolerated. Even after the 500 μ g dose none of the volunteers reported any adverse effects like fever or local oedema. In burn patients the 100 μ g vaccine dose was able to induce an antibody response against the vaccine in 7 of the 8 treated patients. Seroconversion against Tetanus toxoid was observed in the same 7 patients and did also not occur the patient not responding to OprF-OprI vaccination. All patients had a continuous haemodynamic monitoring during the observation period. No signs for an activation of the mediator cascade, like fever or increase of heart rate was observed. Beside the primary vaccination of patients at the time point of the delivery to the hospital, risk groups like soldiers, or patients waiting for an organ transplantation could be prophylactically vaccinated.

In chronically infected CF-patients serum IgG antibodies against outer membrane proteins and LPS can be detected at very high titres. At this stage the patients are obviously unable to clear the pathogen from the airways despite the presence of high levels of antibodies. Since colonisation of the upper respiratory tract seems to precede the pulmonary infection in CF (Johanson et al., 1979; Burns et al., 2001) and IgA is the predominant isotype on the mucosal surface of the upper airways (Pilette et al., 2001) induction of secretory IgA on the airway mucosa may play a critical role for the prevention of adherence and subsequent colonisation of the patho-

genic microorganism (Johanson et al., 1979). Induction of s-IgA antibodies is particularly enhanced by presentation of the antigen at local inductive sites of the mucosa, like the Peyer's patches in the gut or the lymphatic tissue in the nose. Antibody secreting B-cells (ASC) preferentially migrate to effector tissues corresponding to the inductive site (Butcher and Picke, 1996). The expression of mucosal homing receptors is a prerequisite for the induction of mucosal immunity. It should be noted that IgG predominates in the lower airways (Quiding-Jabrink et al., 1997; Kim and Malik, 2003). An ideal vaccine, therefore, would induce high titres of both, local IgA in the upper airways, and IgG in the lower airways. While an oral or intestinal immunisation almost exclusively induces antibody-secreting ASC with a mucosal homing pattern, nasal immunisation was shown to induce a more promiscuous pattern of the ASC with IgA secreting mucosal ASC, and IgG secreting systemic ASC (Kim and Malik, 2003). Nasal vaccination has been shown to induce specific antibodies in the lower respiratory tract (Rudin et al., 1999) and to be protective in mice against pneumococcal infection (Hvalbye et al., 1999). In our mucosal vaccination study we investigated if intranasal application of the OprF-I vaccine would induce the desired s-IgA antibodies in the mucosal of the upper airways secretions together with a systemic IgG response in humans. Supporting the potential of a nasal vaccine, the nasal OprF-I immunisations induced high

levels of systemic and mucosal antibodies of IgA and IgG isotype as obtained in serum and saliva. Since a systemic booster following a mucosal primary was shown to enhance both the systemic and the mucosal immune response, we investigated a further schedule with a systemic booster replacing the second mucosal booster (Muszkat et al., 2000). In our volunteer trial, the systemic booster further enhanced the serum IgG response without compromising the induction of specific s-IgA antibodies in saliva. Moreover, according to preliminary data obtained from induced sputum samples, the systemic booster appears to enhance even the specific IgA levels at the pulmonary airway surface (Baumann et al., 2002).

A mucosal vaccine augmenting the sIgA protection in the respiratory tract may be beneficial also for other patient groups. Patients undergoing major surgery or other severe stress show a compromised oropharyngeal barrier function, a frequent upper airway colonisation with *P. aeruginosa* and an increased risk of a *P. aeruginosa* pneumonia (Johanson et al., 1972,1979).

This patient group has increased considerably during the last decades.

In summary we believe that the results from our phase 1 and 2 studies summarised in this report are in support of a further development of the Met-Ala-(His)₆OprF₁₉₀₋₃₄₂-OprI_{2 1,- 8 3} *Pseudomonas* vaccine to a clinical use in the major risk groups like burn patients and CF patients.

LITERATURE

- Alexander, J.W. and Fisher, M.W.: Immunization against *Pseudomonas* infection after thermal injury. *J. Infect. Dis.* 130, 152-158 (1974).
- Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., and Struhl, K. (eds.): Current protocols in molecular biology 1994-1997 - High efficiency transformation by electroporation. John Wiley and Sons, New York, 1.8.4-1.8.6. (1997).
- Baumann, U., Göcke, K., Hagemann, H.,

- Freihorst, J., and von Specht, B.U.: Mucosal immune response after nasal and systemic vaccination with a recombinant outer membrane protein F and I of *Pseudomonas aeruginosa* in healthy volunteers. *Pediatr. Pulmonol.* 24 (Suppl.), 275 (2002).
- Burns, J.L., Gibson, R.L., McNamara, S., Yim, D., Emerson, J., Rosenfeld, M., Hiatt, P., McCoy, K., Castile, R., Smith, A.L., and Ramsey, B.W.: Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J. Infect. Dis.* 183, 44-452 (2001).
- Butcher, E.C. and Picke, L.J.: Lymphocyte homing and homeostasis. *Science* 272, 60-66 (1996).
- Cohen, J.: Naked DNA points way to vaccines. *Science* 259, 1691-1692 (1993).
- Cryz, S.J. Jr., Fürer, E., Cross, A.S., Wegmann, A., Germanier, R., and Sadoff, J.C.: Safety and immunogenicity of *Pseudomonas aeruginosa* O-polysaccharide-toxin A conjugate vaccine in humans. *J. Clin. Invest.* 80, 51-56 (1987a).
- Cryz, S.J. Jr., Sadoff, J.C., and Fürer, E.: Octavalent *Pseudomonas aeruginosa*-O-polysaccharide-toxin A conjugate vaccine. *Microb. Pathogen.* 6, 75-80 (1987b).
- Cryz, S.J. Jr., Sadoff, C., Ohmann, D., and Fürer, E.: Characterization of the human immune response to a *Pseudomonas aeruginosa* O-polysaccharide-toxin A conjugate vaccine. *J. Lab. Clin. Med.* 111, 701-707 (1988).
- Donnelly, J.J., Ulmer, J.B., and Liu, M.A.: Immunization with polynucleotides. *Immunologist* 2, 20-26 (1994).
- Duchêne, M., Schweizer, A., Lottspeich, F., Krauss, G., Marget, M., Vogel, K., von Specht, B.U., and Domdey, H.: Sequence and transcriptional start site of the *Pseudomonas aeruginosa* outer membrane porin protein F gene. *J. Bacteriol.* 170, 155-162 (1988).
- Duchêne, M., Barron, C., Schweizer, A., von Specht, B.U., and Domdey, H.: *Pseudomonas aeruginosa* outer membrane lipoprotein I gene: molecular cloning sequence, and expression in *Escherichia coli*. *J. Bacteriol.* 171, 4130-4137 (1989).
- Eckhardt, A., Heiss, M.M., Ehret, E., Permanetter, M., Duchêne, M., Domdey, H., and von Specht, B.U.: Evaluation of protective mAbs against *Pseudomonas aeruginosa* outer membrane protein I by C1q binding assay. *Zbl. Bakt.* 1991; 275:100-111.
- Finke, M., Duchêne, M., Eckhardt, A., Domdey, H., and von Specht, B.U.: Protection against experimental *Pseudomonas aeruginosa* infection by recombinant *P. aeruginosa* lipoprotein I expressed in *Escherichia coli*. *Infect. Immun.* 58, 2241-2244 (1990).
- Finke, M., Muth, G., Reichhelm, T., Thoma, M., Duchêne, M., Hungerer, K.D., Domdey, H., and von Specht, B.U.: Protection of immunosuppressed mice against infection with *Pseudomonas aeruginosa* by recombinant *P. aeruginosa* lipoprotein I and lipoprotein I-specific monoclonal antibodies. *Infect. Immun.* 59, 1251-1254 (1991).
- Gabelsberger, J., Knapp, B., Bauernsachs, S., Lenz, U., von Specht, B.U., and Domdey, H.: A hybrid outer membrane protein antigen for vaccination against *Pseudomonas aeruginosa*. *Behring Inst. Mitt.* 98, 302-314 (1997).
- Gallagher, P.G. and Watanakunakorn, Ch.: *Pseudomonas* bacteremia in a community teaching hospital, 1980-1984. *Rev. Infect. Dis.* 11, 846-852 (1989).
- Gilleland, H.E. Jr., Parker, M.G., Matthew, J.M., and Berg, R.D.: Use of purified outer membrane protein F (porin) of *Pseudomonas aeruginosa* as a protective vaccine in mice. *Infect. Immun.* 44, 49-54 (1984).
- Gilleland, H.E. Jr., Gilleland, L.B., and Matthews-Greer, J.M.: Outer membrane protein F preparation of *Pseudomonas aeruginosa* as a vaccine against chronic pulmonary infection with heterologous immunotype strains in rats. *Infect. Immun.* 56, 1017-1022 (1988).
- Gilleland, H.E. Jr., Hughes, E.E., Gilleland, L.B., Matthews-Greer, J.M., and Stacek, J.: Use of synthetic peptides to identify surface-exposed, linear B-cell epitopes within outer membrane protein F of *Pseudomonas aeruginosa*. *Curr. Microbiol.* 31, 279-286 (1995).
- Gilleland, L.B. and Gilleland, H.E. Jr.: Synthetic peptides representing two protective, linear B cell epitopes of outer membrane protein F of *Pseudomonas aeruginosa* elicit whole-cell-reactive antibodies that are functionally pseudomonad specific. *Infect. Immun.* 63:2347-2351 (1995).
- Göcke, K., Baumann, U., Hagemann, H., Gabelsberger, J., Hahn, H., Freihorst, J.,

- and von Specht, B.U.: Mucosal vaccination with a recombinant OprF-I vaccine of *Pseudomonas aeruginosa* in healthy volunteers: Comparison of a systemic vs. a mucosal booster schedule. *FEMS Immunol. Med. Microbiol.* 37, 167-171 (2003).
- Gordon, S.M., Serke, J.M., Keys, T.F., Ryan, T., Fatica, C.A., Schmitt, S.K., Borsh, J.A., Cosgrove, D.M., and Yared, J.P.: Secular trends in nosocomial bloodstream infections in a 55-bed cardiothoracic intensive care unit. *Ann. Thorac. Surg.* 65, 95-100 (1998).
- Griffith, S.J., Nathan, C., Selander, R.K., Chamberlin, W., Gordon, S.T., Kabins, S., and Weinstein, R.A.: The epidemiology of *Pseudomonas aeruginosa* in oncology patients in a general hospital. *J. Infect. Dis.* 160, 1030-1036 (1989).
- Hanberger, H., Hoffmann, M., Lindgren, S., and Nilson, L.E. High incidence of antibiotic resistance among bacteria in 4 intensive care units at a university hospital in Sweden. *Scand. J. Infect. Dis.* 29, 602-614 (1997).
- Hancock, R.E.W.: Intrinsic antibiotic resistance of *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 18, 653-656 (1986).
- Hancock, R.E.W. and Wong, R.: Potential of protein OprF of *Pseudomonas* in bivalent vaccines. *Behring Inst. Mitt.* 98, 283-290 (1997).
- Hoiby, N., Krogh, J.H., Moser, C., Song, Z., Ciofu, O., and Kharazmi, A.: *Pseudomonas aeruginosa* and the *in vitro* and *in vivo* biofilm mode of growth. *Microbes Infect.* 3, 23-35 (2001).
- Holder, I.A.: *Pseudomonas* immunotherapy. *Serodiagn. Immunother.* 2, 7-16 (1988).
- Holzheimer, R.G., Quoika, P., Pätzmann, D., and Füssle, R.: Nosocomial infections in general surgery: Surveillance report from a German University. *Clin. Infect.* 18, 219-225 (1990).
- Hong, Y.Q. and Ghebrehiwet, B.: Effect of *Pseudomonas aeruginosa* elastase and alkaline protease on serum complement and isolated components C1q and C3. *Clin. Immun. Immunopath.* 62, 1333-1338 (1992).
- Hsueh, P.R., Teng, L.J., Chen, Y.C., Ho, S.W., and Lu, K.T.: Persistence of a multidrug-resistance *Pseudomonas aeruginosa* clone in an intensive care burn unit. *J. Clin. Microbiol.* 2, 1347-1351 (1998).
- Hvalbye, B.K., Aaberge, I.S., Lovik, M., and Haneberg, B.: Intranasal immunization with heat-inactivated *Streptococcus pneumoniae* protects mice against systemic pneumococcal infection. *Infect. Immun.* 67, 4320-4325 (1999).
- Johanson, W.G. Jr., Pierce, A.K., Sanford, J.P., and Thomas, G.D. Nosocomial respiratory infections with gram-negative bacilli. The significance of colonization of the respiratory tract. *Ann. Intern. Med.* 77, 701-706 (1972).
- Johanson, W.G. Jr., Woods, D.E., and Chaudhuri, T.: Association of respiratory tract colonization with adherence of gram-negative bacilli to epithelial cells. *J. Infect. Dis.* 139, 667-673 (1979).
- Jones, R.J., Roe, E.A., and Gupta, J.L.: Controlled trials of a polyvalent *Pseudomonas* vaccine in burns. *Lancet* 2, 977-983 (1979).
- Jung, S.B., Ahn, B.Y., Kim, Y.H., Kim, J.J., Kim, D.K., Kim, I.S., Yoon, S.M., Nam, S.W., Kim, H.S., and Park, W.J.: Immunization of burn-patients with a *Pseudomonas aeruginosa* outer membrane protein vaccine elicits antibodies with protective efficacy. *Vaccine* 18, 1952-1961 (2000).
- Kim, D.K., Kim, J.J., Kim, J.H., Woo, Y.M., Kim, S., Yoon, D.W., Choi, C.S., Kim, I., Park, W.J., Lee, N., Jung, S.B., Ahn, B.Y., Nam, S.W., Yoon, S.M., and Choi, W.J.: Comparison of two immunization schedules for a *Pseudomonas aeruginosa* outer membrane proteins vaccine in burn patients. *Vaccine* 19, 1274-1283 (2000).
- Kim, K.J. and Malik, A.B.: Protein transport across the lung epithelial barrier. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 284, L247-259 (2003).
- Koch, C. and Hoiby, N.: Pathogenesis of cystic fibrosis. *Lancet* 341, 1065-1069 (1993).
- Korvick, J.A., Marsh, J.W., Starzl, T.E., and Yu, V.L.: *Pseudomonas aeruginosa* bacteremia in patients undergoing liver transplantation: An emerging problem. *Surgery* 109, 62-68 (1991).
- Kraehenbuhl, J.P. and Neutra, M.R.: Molecular and cellular basis of immune protection of mucosal surfaces. *Physiol. Rev.* 72, 853-879 (1992).
- Larbig, M., Mansouri, E., Freihorst, J., Tümmler, B., Kohler, G., Domdey, H., Knapp, B., Hungerer, K.D., Hundt, E.,

- Gabelsberger, J., and von Specht, B.U.: Safety and immunogenicity of an intranasal *Pseudomonas aeruginosa* hybrid outer membrane protein F-I vaccine in human volunteers. *Vaccine* 19, 2291-2297 (2001).
- Mansouri, E., Blome-Eberwein, S., Knapp, B., Hungerer, K.D., Germann, G., and von Specht, B.U.: Clinical study to assess the immunogenicity and tolerability of a recombinant *Pseudomonas aeruginosa* high efficiency transformation by electroporation. *Curr. Protocols Mol. Biol.* 1994-1997 (1997).
- Mansouri, E., Gabelsberger, J., Knapp, B., Hundt, E., Lenz, U., Hungerer, K.D., Gilleland, H.E. Jr., Staczek, J., Domdey, H., and von Specht, B.U.: Safety and immunogenicity of a *Pseudomonas aeruginosa* hybrid outer membrane protein F-I vaccine in human volunteers. *Infect. Immun.* 67, 1461-1470 (1999).
- Matthews-Greer, J.M. and Gilleland, H.E. Jr.: Outer membrane protein F (porin) preparation of *Pseudomonas aeruginosa* as a protective vaccine against heterologous immunotype strains in a burned mouse model. *J. Infect. Dis.* 155, 1282-1291 (1987).
- McGhee, J.R., Czerkinsky, C., and Mestecky, J.: Mucosal vaccines: An overview. In: *Mucosal immunology* (Eds.: Ogra, P.L., Mestecky, J., Lamm, M.E., Strober, W., Bienenstock, J., and McGhee, J.R.). Academic Press, San Diego, California, 844-850 (1999).
- McManus, A.T., Mason, A.D. Jr., McManus, W.F., and Pruitt, B.A. Jr.: Twenty-five year review of *Pseudomonas aeruginosa* bacteremia in a burn center. *Eur. J. Clin. Microbiol.* 4, 219-223 (1985).
- Miller, J.M., Spilsbury, J.F., Jones, R.J., Roe, E.A., and Lowbury, E.J.L.: A new polyvalent *Pseudomonas* vaccine. *J. Med. Microbiol.* 10, 19-27 (1977).
- Muszkat, M., Yehuda, A.B., Schein, M.H., Friedlander, Y., Naveh, P., Greenbaum, E., Schlesinger, M., Levy, R., Zakay-Rones, Z., and Friedman, G.: Local and systemic immune response in community-dwelling elderly after intranasal or intramuscular immunization with inactivated influenza vaccine. *J. Med. Virol.* 61, 100-106 (2000).
- Mutharia, L.M., Nicas, T.L., and Hancock, R.E.W.: Outer membrane proteins of *P. aeruginosa* serotype strains. *J. Infect. Dis.* 146, 770-779 (1982).
- Pennington, J.E.: *Pseudomonas aeruginosa* pneumonia and other respiratory tract infections. In: *Pseudomonas aeruginosa* infections and treatment (Ed.: Baltch, A.). Marcel Dekker Inc., New York, 159-181 (1994).
- Pier, G.B.: Safety and immunogenicity of high-molecular weight polysaccharide vaccine from immunotype 1 *Pseudomonas aeruginosa*. *J. Clin. Invest.* 69, 303-308 (1982).
- Pier, G.B.: *Pseudomonas aeruginosa* surface polysaccharide vaccines. In: *Pseudomonas aeruginosa: New therapeutic approaches from basic research*, Vol. 36 (Eds.: Speert, D.P. and Hancock, R.E.W.). S. Karger, Basel, 157-167 (1985).
- Pier, G.B., Des Jardin, D., Grout, M., Garner, C., Bennet, S.E., and Pekoe, G.: Human immune response to *Pseudomonas aeruginosa* mucoid exopolysaccharide (alginate) vaccine. *Infect. Immun.* 62, 3972-3979 (1994).
- Pier, G.B.: Rational for development of immunotherapies that target mucoid *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *Behring Inst. Mitt.* 98, 350-360 (1997).
- Pilette, C., Ouadriri, Y., Godding, V., Vaerman, J.P., and Sibille, Y.: Lung mucosal immunity: Immunoglobulin-A revisited. *Eur. Respir. J.* 18, 571-588 (2001).
- Price, B.M., Barten Legutki, J., Galloway, D.R., von Specht, B.U., Gilleland, L.B., Gilleland, H.E. Jr., and Staczek, J.: Enhancement of the protective efficacy of an oprF DNA vaccine against *Pseudomonas aeruginosa*. *FEMS Immunol. Med. Microbiol.* 33, 89-99 (2002).
- Pruitt, B.A. Jr., Colonel, M.C., and McManus, A.T.: Opportunistic infections in severely burned patients. *Am. J. Med.* 30, 146-154 (1984).
- Pruitt, B.A. Jr., McManus, A.T., Kim, S.H., and Goodwin, C.W.: Burn wound infections: Current status. *World J. Surg.* 22, 135-145 (1998).
- Quiding-Jabrink, M., Nordstrom, I., and Granstrom, G.: Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentaliza-

- tion of effector B cell responses. *J. Clin. Invest.* 99, 1281-1286 (1997).
- Rudin, A., Riise, G.C., and Holmgren, J.: Antibody responses in the lower respiratory tract and male urogenital tract in humans after nasal and oral vaccination with cholera toxin B subunit. *Infect. Immun.* 67, 2884-2890 (1999).
- Srikumar, R., Kon, T., Gotoh, N., and Poole, K.: Expression of *Pseudomonas aeruginosa* multidrug efflux pumps MexA-MexB-OprM and MexC-MexD-OprJ in a multidrug-sensitive *Escherichia coli* strain. *Antimicrob. Agents Chemother.* 42, 65-71 (1988).
- Tassios, P.T, Gennimata, V., Maniatis, A.N., Fock, C., and Legakis, N.J.: Emergence of multidrug resistance in ubiquitous and dominant *Pseudomonas aeruginosa* serogroup O:11. The Greek *Pseudomonas aeruginosa* Study Group. *J. Clin. Microbiol.* 36, 897-901 (1998).
- Tobiasen, J., Hiebert, J., and Edlich, R.: Prediction of burn mortality. *Surg. Gynecol. Obstet.* 154, 711-714 (1982).
- Toth, A., Schödel, F., Duchêne, M., Massarrat, K., Blum, B., Schmitt, A., Domdey, H., and von Specht, B.U.: Protection of immunosuppressed mice against translocation of *Pseudomonas aeruginosa* from the gut by oral immunization with recombinant *Pseudomonas aeruginosa* outer membrane protein I expressing *Salmonella dublin*. *Vaccine* 12, 1215-1221 (1994).
- von Specht, B.U., Strigl, G., Ehret, W., and Brendel, W.: Protective effect of an outer membrane vaccine against *Pseudomonas aeruginosa* infection. *Infection* 15, 408-412 (1987).
- von Specht, B.U., Knapp, B., Muth, G., Bröker, M., Hungerer, K.D., Diehl, K.D., Massarrat, K., Seemann, A., and Domdey, H.: Protection of immunocompromised mice against lethal infection with *P. aeruginosa* by active or passive immunization with recombinant *P. aeruginosa* outer membrane protein F and outer membrane protein I fusion proteins. *Infect. Immun.* 63, 1855-1861 (1995).
- von Specht, B.U., Gabelsberger, J., Knapp, B., Hundt, E., Schmidt-Pilger, H., Bauernsachs, S., Lenz, U., and Domdey, H.: Immunogenic efficacy of differently produced recombinant vaccines candidates against *Pseudomonas aeruginosa* infections. *J. Biotechnol.* 83, 3-12 (2000).