

# Prophylaxis and therapy of *Pseudomonas aeruginosa* infection in cystic fibrosis and immunocompromised patients

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## Abstract

*Pseudomonas aeruginosa* is an opportunistic bacterium responsible for chronic lung infection in cystic fibrosis patients, as well as nosocomial infections in immunocompromised patients. An O-polysaccharide-toxin A conjugate vaccine was evaluated for prophylaxis of *P. aeruginosa* in cystic fibrosis patients. Vaccination proved to be useful in preventing and/or delaying infection. Fully human monoclonal antibodies (mAb) against *P. aeruginosa* O-polysaccharides were developed for the treatment of immunocompromised patients in whom active immunoprophylaxis is not applicable. Characterisation of the mAb revealed high antigen specificity and avidity, as well as excellent efficacy in relevant in vitro and in vivo systems, permitting future clinical evaluation.

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**Keywords:** *Pseudomonas aeruginosa*; Cystic fibrosis; Vaccine; Human monoclonal antibodies

## 1. Introduction: *Pseudomonas aeruginosa* as an opportunistic pathogen

*Pseudomonas aeruginosa* is a ubiquitous Gram-negative environmental bacterium found in fresh water and soil. It is a classical opportunistic pathogen that does not normally pose a threat to the immunocompetent host, who clears it by means of opsonising antibodies and phagocytosis. However, a specific group of individuals at risk of contracting *P. aeruginosa* infection is patients suffering from cystic fibrosis (CF) [1,2]. The CF lung is an ideal environment for the growth of *P. aeruginosa*, reflected in the high prevalence of chronic pseudomonal lung infection in these patients. Moreover, upon chronic infection of the lung *P. aeruginosa* tends to change from a rough to a mucoid phenotype [3]. Due to the ineffective penetration of components of the immune system into the slime capsule the bacterium is capable to evade the immune system. Since the immune system of CF patients otherwise functions normally, active vaccination is a feasible strategy for prevention of *Pseudomonas* infection.

*P. aeruginosa* is also an important pathogen in individuals whose phagocytic system is compromised. These include

neutropenic patients undergoing immunosuppressive treatment, those with burns, and those artificially ventilated in an intensive care unit (ICU) [4–6]. Acute infections in these patients can have rapid and dramatic courses. Thus, often there is no time to establish endogenous immunity through active vaccination. Furthermore, due to their immunosuppressed status these patients are not able to mount effective immune responses on their own. For these reasons, passive immunotherapy represents an attractive alternative approach for treatment.

## 2. Active immunisation of CF patients against *P. aeruginosa* infection

At age 20, up to 80% of CF patients suffer from chronic lung infection with *P. aeruginosa* [7]. In a reaction to initial infection, the innate and adaptive arms of the immune system mount vigorous responses against *P. aeruginosa*. However, these are ineffective in eradicating the bacteria from the lungs, but lead to massive inflammation, associated with destruction of lung tissue, and ultimately loss of lung function [1,8]. Despite considerable progress in treatment with antibiotics in recent years, it has proved virtually impossible to eradicate the organism after its establishment in the lower respiratory

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tract. Additionally, antibiotic resistance of *P. aeruginosa* frequently interferes with successful treatment [9]. Effective prophylaxis against *P. aeruginosa* infection through active vaccination could further improve the clinical management of CF.

### 2.1. Development of a prophylactic anti-*Pseudomonas* conjugate vaccine

Since chronic infection with *P. aeruginosa* represents a problem for virtually all CF patients, prophylactic treatment of the entire patient group should be useful. Generally, any significant virulence factor of *P. aeruginosa* might be targeted for active immunisation. These may include outer membrane proteins, flagellae, pili and secreted products or translocation proteins of type III secretion systems (such as PcrV) [10]. High-affinity antibodies to lipopolysaccharides (LPS) have also been shown to afford excellent protection against *P. aeruginosa* infection in pre-clinical and clinical evaluations [10]. However, despite the fact that CF patients with intermittent or chronic infection have elevated antibody titres against *P. aeruginosa* LPS, these patients are apparently not adequately protected by their infection-induced immune response [11]. The lack of protective capacity may be explained by the low affinity and low effector function of infection-induced anti-LPS antibodies [12]. Coupling polysaccharide components to proteins for immunisation results in the induction of high-affinity antibodies to the polysaccharide components. To exploit this concept, we developed a conjugate vaccine comprising the eight most prevalent LPS-serotypes of *P. aeruginosa* (IATS-1, 2, 3, 4, 6, 10, 11, 16) coupled to toxin A of *P. aeruginosa* (Fig. 1). We hypothesised that this method of presenting O-polysaccharides to the immune system might lead to induction of antibodies with a high affinity and effector function, and thus to a high degree of protection in CF patients.

### 2.2. Clinical experience

After initial safety evaluation in healthy adults [13] a phase I clinical trial was conducted in CF patients [14]. Following initial vaccination these patients continued to receive booster

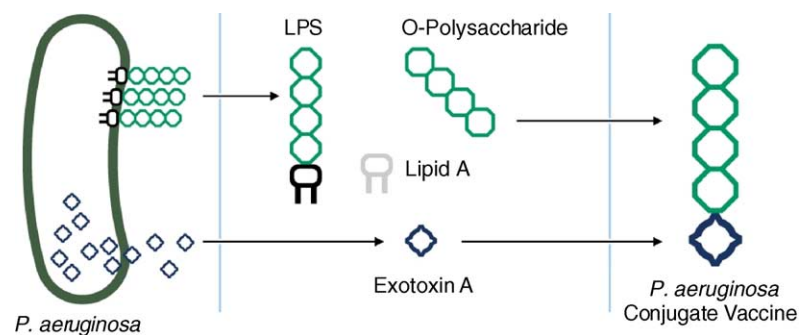


Fig. 1. Synthesis of an octavalent anti-*Pseudomonas* polysaccharide conjugate vaccine. Detoxified O-polysaccharides of eight different serotypes are individually coupled to detoxified exotoxin A of *P. aeruginosa*. The eight individual constructs are mixed in equal portions to constitute the final octavalent conjugate vaccine.

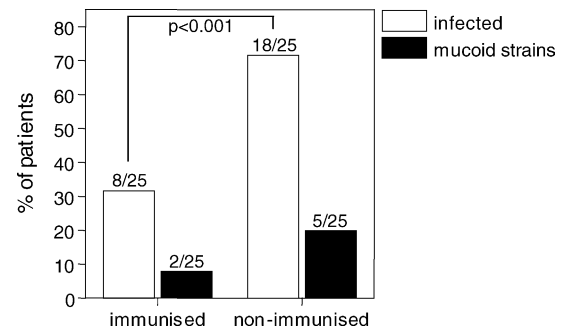


Fig. 2. *P. aeruginosa* infection in immunised vs. non-immunised CF patients. CF patients were immunised at yearly intervals for 10 years (“immunised”) and compared to a matched control group (“non-immunised”). Rate of infection with *P. aeruginosa* (white bars) and emergence of mucoid *P. aeruginosa* among chronically infected patients (black bars) is shown.  $p$  was calculated using Kaplan–Meyer analysis described in [17].

immunisations at yearly intervals, and were compared with matched controls over a prolonged period [15,16]. Vaccinees and controls, except for yearly vaccinations over a total period of 10 years, underwent the same management at a single paediatric CF centre in Bern, Switzerland, throughout the study. Recently, we reported [17] the results of a retrospective review of clinical records after 10 years of follow-up (1989–2001). We showed a significantly reduced incidence of chronic infection with *P. aeruginosa* in the immunised group. Only 8/25 (32%) patients became chronically infected with *P. aeruginosa* compared to 18/25 (72%) patients in the control group (Fig. 2). Likewise, the emergence of mucoid strains among chronically infected patients was lower in the immunised group (Fig. 2). Time to infection in the immunised group was longer compared to the non-immunised group. These findings were also reflected in the lower frequency of *P. aeruginosa* in sputa/throat swabs of immunised patients compared to the control group (20.8% versus 49.4%). As a consequence, immunisation over a period of 10 years led to preservation of lung function, particularly in older patients. Initial studies indicated significantly higher binding affinity of vaccine-induced compared to infection-induced anti-LPS serum IgG antibodies [12]. Similar results were obtained in further analyses showing that mean affinity of vaccine-induced serum IgG antibodies was about 1 log higher than

that of infection-induced IgG antibodies ([15] and Zuercher et al., manuscript in preparation). These findings confirm our initial hypothesis that the protective capacity of anti-LPS antibodies is strongly linked to affinity.

Effective prophylaxis against chronic *P. aeruginosa* infection/colonisation of CF patients would be an important addition to current antibiotic treatment. Our experiences with an octavalent polysaccharide conjugate vaccine indicate safety for long-term use, immunogenicity and clinical efficacy. To validate these data, a multinational, double-blind, placebo controlled phase III clinical trial is currently in progress.

### 3. Passive immunisation against nosocomial infection with human monoclonal antibodies

Hospital-acquired (nosocomial) infections are increasingly responsible for serious secondary illness in the hospital environment. *P. aeruginosa* is a leading cause of nosocomial infection, along with coagulase-negative staphylococci, *Staphylococcus aureus* and *Enterococcus* spp. [4,6]. These bacterial species are often (and increasingly) resistant or multi-resistant to antibiotics [18]. Immunocompromised individuals, including burn victims, intubated patients in ICU, cancer and AIDS patients, as well as patients undergoing organ transplantation are at particularly high risk of contracting nosocomial infections. Because of their compromised immune status and the acute course of infection, active vaccination is usually not applicable in these patients. Therefore, there is a need for alternative therapeutic tools to treat nosocomial infection. In this context, the use of human monoclonal antibodies (mAb) for passive immunotherapy is a promising approach.

mAb can be produced by different techniques. Hybridoma technology is a well-established method for this purpose. B cells of desired specificity can be elicited by immunisation with an antigen of choice, and immortalised by fusion with a myeloma cell-line [19]. If generated in another species, mAb have to be humanised by CDR-grafting or phage display technology to enable clinical use. However, it is known that, for example, murine antibodies against bacterial LPS often recognise other epitopes than human antibodies [20]. Therefore, humanised mouse-antibodies might not have the specificities essential to confer protection in humans. Furthermore, polysaccharides (including LPS) are T cell independent antigens, and antibodies induced in response to them are mostly of the IgM isotype. The major defence mechanisms against bacterial infection are complement-activated killing and complement mediated opsonophagocytosis. Thus, IgM antibodies have several advantages as therapeutic tools. Firstly, due to their pentameric form, they possess 10 binding sites for the LPS antigen, and can bind the antigen with high avidity. Secondly, as outlined above, IgM is by its nature a very effective complement activator [21]. Nowadays human mAb are often generated, using recombinant

technology, such as phage display repertoire cloning. Whereas, the isolation of LPS-specific protective antibodies can indeed easily be achieved in these systems, the recombinant production of human IgM antibodies is still an unsolved problem. An alternative method that might be used to overcome these limitations is generation of human hybridomas by direct fusion of human antigen-specific B cells with a suitable fusion-partner for immortalisation [22].

#### 3.1. Generation of anti-*Pseudomonas* human mAb

There have been various attempts to generate human mAb against LPS moieties of *P. aeruginosa* [23–25]. However, many of them resulted in mAb that lacked effector functions and were not protective. We had observed that immunisation with our octavalent O-polysaccharide conjugate vaccine-induced protective immune responses in humans [13–17]. Therefore, we used lymphocytes from healthy volunteers actively immunised with this vaccine for the generation of human hybridomas. Antigen-specific B cells from peripheral blood were enriched by panning on plates coated with LPS prior to immortalisation through fusion with our proprietary non-secreting fusion partner, a mouse-human heteromyeloma (Fig. 3). With these techniques, we generated a panel of human mAb against the most frequent serotypes of *P. aeruginosa*, as well as a wide range of other Gram-negative bacteria, using other vaccines [22,26,27]. One candidate anti-*Pseudomonas* mAb has been selected for further development and evaluation in clinical trials. Biosafety evaluation revealed conformity with requirements to initiate clinical trials according to current guidelines. The cell line had excellent antibody production capacity, and was easily adapted to serum-free conditions and scaled-up for use in perfusion fermentors.

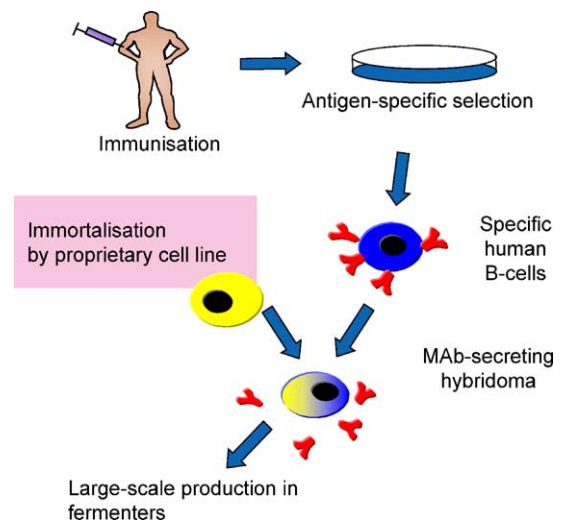


Fig. 3. Generation of human hybridomas. Simplified schematic presentation of the process for human hybridoma generation.

Table 1  
Comparison of avidity of different anti-LPS mAb

Antibody	Species	Isotype	IATS-specificity	Avidity <sup>a</sup> ( $\times 10^6 \text{ M}^{-1}$ )
M410	Human	IgM	2/16	750
NA3-24	Human	IgA1	2/16	46
37T3	Mouse	IgG3	2/16	14
4-10K	Human	IgM	11	44
63T2	Mouse	IgM	11	0.91
99T2	Mouse	IgG2b	11	0.63

<sup>a</sup> Avidity for LPS of IATS serotypes 2/16 or 11 was determined by inhibition ELISA and is expressed as the reciprocal LPS concentration in suspension resulting in 50% inhibition of mAb binding to solid phase LPS.

### 3.2. Characterisation and biological effector functions of anti-*Pseudomonas human mAb*

Characterisation of various anti-*Pseudomonas* mAb generated and produced in our system showed exclusive specificity for LPS of one serotype of *P. aeruginosa*. Importantly, not only was purified, plate-bound LPS recognised, but the mAb also reacted with LPS on the surface of clinical isolates of *P. aeruginosa*; an important prerequisite for clinical use. We had observed previously that the protective capacity of anti-*Pseudomonas* antibodies induced by active vaccination was linked to high affinity [12]. Therefore, we performed avidity measurement of our human IgM mAb, using an inhibition ELISA assay. As shown in Table 1, the highest avidity for LPS found was for human IgM, compared to other human isotypes (16-fold higher), as well as for murine mAb (48–70-fold higher) [28].

As mentioned above, the major mechanism for elimination of bacteria is opsonisation, followed by complement mediated phagocytosis by neutrophils and macrophages (opsonophagocytosis). We developed an in vitro opsonophagocytosis assay based on flow cytometry for read-out (Fig. 4A) to measure the capacity of our human anti-*P. aeruginosa* mAb to mediate opsonophagocytosis. In this assay, mAb are incubated with fluorescently (FITC) labelled *P. aeruginosa* in the presence or absence of complement, followed by incubation with macrophage-like cells, derived from the pro-myelotic cell line HL-60. As shown in Fig. 4B, we observed a concentration-dependent uptake of opsonised FITC-conjugated bacteria by the macrophages. This process was complement dependent, since in the absence of complement no significant phagocytosis was observed. Furthermore, phagocytosis was serotype-specific, and the mAb exhibited phagocytosis at less than 0.01  $\mu\text{g/ml}$  (Fig. 4B). This in vitro test is a good indicator for the in vivo effector function of the mAb.

*P. aeruginosa* is not a classical pathogen for mice; nevertheless, there are in vivo challenge models in mice. We used the murine burn wound sepsis model [29] for pre-clinical efficacy assessment in vivo. Our experiments revealed excellent protective capacity of human mAb at doses of 1–5  $\mu\text{g}/\text{mouse}$  for prophylactic treatment of *P. aeruginosa*-induced sepsis

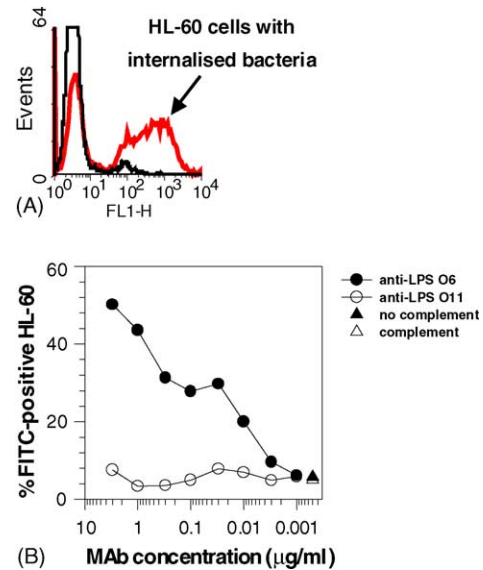


Fig. 4. Opsonophagocytosis assay with HL-60 cells. (A) Complement and antibody dependent phagocytosis of fluorescently labelled *P. aeruginosa* by HL-60 cells visualised by flowcytometry (red line). Incubation of HL-60 cells with fluorescently labelled *P. aeruginosa* and antibody only does not result in phagocytosis (black line); (B) Titration of LPS-specific (closed circles, anti-IATS-6) and non-specific (open circles, anti-IATS-11) mAb on FITC-conjugated *P. aeruginosa* serotype IATS-6. Open and closed triangles depict assay controls. Phagocytosis is serotype-specific and complement-dependent.

[22]. In these experiments mAb was administered i.v. 3–24 h before challenge. Typically, three different doses of bacteria were used for challenge, depending on the virulence of individual strain. Furthermore, we showed full therapeutic efficacy by treatment of mice up to 6 h after the onset of sepsis. Administration of mAb 18 h after challenge still had some protective effect but due to the rapid course of disease in mice with death occurring usually within 24 h after challenge no later time-points for evaluation of therapeutic efficacy were applicable.

## 4. Conclusion

In conclusion, the rise of antibiotic-resistance in nosocomial bacteria warrants development of new therapeutic tools to combat these infections. The most frequent victims of such infections are immunocompromised patients in ICU. As these patients cannot be actively immunised, the use of human mAb directed against the bacteria is a promising approach for treatment. One great advantage of mAb in comparison to antibiotics is the fact that there is no risk of generating resistance against the mAb. The generation, characterisation and production of biologically active and fully human mAb has been developed to a robust technology platform, using our proprietary fusion partner. One anti-*Pseudomonas* mAb has been produced under GMP conditions, and will be tested in a phase I clinical trial in 2004.

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