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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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
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, pilus 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 (IA TS-1, 2, 3, 4, 6, 10, 11, 16) coupled to exotoxin 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 1 clinical trial was conducted in CF patients [14]. Following initial vaccination these patients continued to receive booster immunisations at yearly intervals, and were compared with matched controls over a prolonged period [15,16]. Vaccinates 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...
what 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 antibo-
dies is strongly linked to affinity.

Effective prophylaxis against chronic *P. aeruginosa* in-
fec tion/colonisation of CF patients would be an important addi-
tion to current antibiotic treatment. Our experiences with an octavalent polysaccharide conjugate vaccine indi-
cate safety for long-term use, immunogenicity and clinical ef-
cacy. 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 increas-
ingly responsible for serious secondary illness in the hospi-
tal environment. *P. aeruginosa* is a leading cause of noso-
comial infection, along with coagulase-negative staphylo-
cocci, *Staphylococcus aureus* and *Enterococcus* spp. [4,6].
These bacterial species are often (and increasingly) resis-
tant or multi-resistant to antibiotics [18]. Immunocompro-
ised individuals, including burn victims, intubated patients in
ICU, cancer and AIDS patients, as well as patients un-
dergoing organ transplantation are at particularly high risk
of contracting nosocomial infections. Because of their com-
promised 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 dis-
play 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. Fur-
thermore, polysaccharides (including LPS) are T cell inde-
pendent antigens, and antibodies induced in response to them
are mostly of the IgM isotype. The major defence mech-

anisms 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 bind-
ing 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]. Now-
a-days human mAb are often generated, using recombin-
tation technology, such as phage display repertoire cloning.
Whereas, the isolation of LPS-specific protective antibod-
ies 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 hu-
man 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 immunisa-
tion 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 periph-
eral blood were enriched by panning on plates coated with
LPS prior to immortalisation through fusion with our pro-
prietary non-secreting fusion partner, a mouse-human het-
 eromyeloma (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 develop-
ment and evaluation in clinical trials. Biosafety evaluation
revealed conformity with requirements to initiate clinical tri-
als according to current guidelines. The cell line had excel-
lent 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.](image-url)
Table 1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Species</th>
<th>Isotype</th>
<th>IA TS-specificity</th>
<th>Avidity $^a$ ($\times 10^6 M^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M410</td>
<td>Human</td>
<td>IgM</td>
<td>2/16</td>
<td>750</td>
</tr>
<tr>
<td>NA3-24</td>
<td>Human</td>
<td>IgA1</td>
<td>2/16</td>
<td>46</td>
</tr>
<tr>
<td>37T3</td>
<td>Mouse</td>
<td>IgG3</td>
<td>2/16</td>
<td>14</td>
</tr>
<tr>
<td>4-10K</td>
<td>Human</td>
<td>IgM</td>
<td>11</td>
<td>0.91</td>
</tr>
<tr>
<td>63T2</td>
<td>Mouse</td>
<td>IgM</td>
<td>11</td>
<td>0.63</td>
</tr>
</tbody>
</table>

$^a$ Avidity for LPS of IA TS 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 μg/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 μg/mouse for prophylactic treatment of P. aeruginosa-induced sepsis.

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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References


