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Vaccine strategies against cystic fibrosis pathogens

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ABSTRACT

A great number of cystic fibrosis (CF) pathogens such as *Pseudomonas aeruginosa*, the *Burkholderia cepacia* and the *Mycobacterium abscessus* complex raised difficult therapeutic problems due to their intrinsic multi-resistance to numerous antibiotics. Vaccine strategies represent one of the key weapons against these multi-resistant bacteria in a number of clinical settings like CF. Different strategies are considered in order to develop such vaccines, linked either to priming the host response, or by exploiting genomic data derived from the bacterium. Interestingly, virulence factors synthesized by various pathogens might serve as targets for vaccine development and have been, for example, evaluated in the context of CF.

Keywords: cystic fibrosis, vaccine, *Mycobacterium abscessus*, *Pseudomonas aeruginosa*, *Burkholderia* spp.

Cystic fibrosis and microbial lung infections.

Cystic fibrosis (CF) is a disease which arises from a Mendelian defect due to a series of mutations in the *cftr* gene encoding the Cl^- channel[1]. The resulting flaw in this protein is responsible for increasing the viscosity of the mucus, which promotes the accumulation and the attachment of bacteria to mucins. Chronic inflammation[2] and early bacterial infection maintain a vicious circle and are each responsible for the lung damage which ensues. Lung infections in CF patients represent the most frequent but also the more serious manifestations since they are responsible for more than 90% of CF patient deaths[3]. The microorganisms that may infect the respiratory system are bacteria, fungi and viruses. Bacterial colonization occurs very early in the natural history of the disease[4]. The first causative organisms are *Haemophilus influenzae* and *Staphylococcus aureus*. *S. aureus* is usually the first detected[5] and its prevalence is rising[6]. Affinity of *S. aureus* for CF mucus contributes to persistent colonization and progressive pulmonary damage increasing the potential for further infections to set in, for example *Pseudomonas* spp. [5]. *Pseudomonas aeruginosa* colonization arises several months to several years after. Finally, several bacterial complexes are found responsible for severe infections in CF, in addition to be the most difficult to treat: the *Burkholderia cepacia* complex (Bcc) and the *Mycobacterium abscessus* complex, which has emerged recently as a threat in CF patients, and may present with *Mycobacterium avium*, the major non-tuberculous mycobacterium (NTM) present in CF lungs with a significant prevalence[7,8].

Opportunistic pathogens becoming untreatable weapons in CF patients

P. aeruginosa is the environmental opportunistic pathogen in CF patients. It is the most commonly isolated bacterium that infects individuals with CF, with colonization and chronic infections that may affect up to 80% of adult CF patients[9]. *P. aeruginosa* establishes a chronic

endobronchial infection which impacts on morbidity and mortality of CF patients. *P. aeruginosa* is also notable for its resistance to antibiotics, making it therefore a difficult to treat pathogen, which, once acquired, is rarely, if ever, eradicated. In addition, *P. aeruginosa* frequently colonized CF lungs as a biofilm, which reduces the patient's immune response and access by antibiotics [10]. A second opportunistic pathogen, represented as a complex is the *Burkholderia cepacia* complex (Bcc). It is composed of 18 species that are able to cause opportunistic and lethal infections CF patients[11]. The two most clinically relevant species are *Burkholderia cenocepacia* and *Burkholderia multivorans*[12]. These environmental, intracellular and biofilm-forming bacteria are extremely antibiotic resistant organism[12]. Bcc infections are rarely cleared from CF patients once they are colonized, as observed in *P. aeruginosa* infections. The third antibiotic-resistant bacterium found in CF patients with frequency between 3 to 7% [7,13] is *Mycobacterium abscessus*. It is a rapidly growing mycobacterium also existing as a complex: the *Mycobacterium abscessus* complex[14], with two subspecies *M. abscessus abscessus* and *M. abscessus bolletii* respectively. *M. abscessus* is, within the group of rapid-growers, responsible for a broad spectrum of diseases in humans. Lung infections are frequent, with CF patients particularly susceptible [7,13,15], in addition to muco-cutaneous infections often of nosocomial origin[16]. Recent reports of human-to-human transmission in the context of CF care have been described[17,18]. *M. abscessus* raises very challenging therapeutic issues because of its natural resistance to most available antibiotics[19,20]. Severe, even fatal, infections in CF patients have been described due to therapeutic deadlock[21]. *M. abscessus* infection might represent a contraindication for lung transplantation in several countries[22], leaving CF patients without therapeutic options.

As such, antibiotic treatment exemplifies a clear challenge now faced with these opportunistic pathogens. We demonstrated for example a significant link between previous intravenous antibiotic courses and the isolation of *M. abscessus* in CF patient lungs, underlining the role

of broad-spectrum antimicrobial therapy in the emergence of *M. abscessus* disease[23]. And this is true for the continuous emergence of resistant *P. aeruginosa* or Bcc due to the repeated antibiotic therapeutic regimens given to CF patients[24]. Emergence of multi-resistant bacteria leads to therapeutic impasses with severe and fatal infections[24].

Vaccine approaches

Pathogens can be divided into two groups according to whether vaccines exist against them or not. However, no human vaccine has been developed so far against the antibiotic-resistant pathogens described above. As such, the development of prophylactic or therapeutic vaccines is of extreme importance when confronted with bacteria of this high resistance..

When defining an appropriate target, making the correct choice and how such tools can be developed is a long and tedious process. Development of vaccine targets can profit from genome sequence comparisons[25], and, using a process known as reverse vaccinology, might introduce a novel strategy to identify target antigens that might serve as potential vaccines [25]. We chose this strategy for the development of a first vaccine against *M. abscessus*[26] (see below). Indeed, the presence of specific genes in opportunistic pathogen genomes that are absent from saprophytic bacterial genomes belonging to the same genus represents the key to unravel virulence factors that can then be targeted using a vaccine approach. A better understanding of the "virulence" genes contributes greatly to the development of new control strategies against these microorganisms. In addition, the choice of a virulence factor as a vaccine target has been shown to be relevant in the scientific literature [25].

Vaccination can then be performed using recombinant proteins when expression and purification are possible; or using the expression of plasmid DNA with modified eukaryotic gene sequences, as has been performed with *M. abscessus*[26]. In fact, in the different vaccine strategies, DNA vaccines exemplify one of these new strategies and has been used successfully in the context of infectious diseases[27]. Over the last 15 years, DNA vaccines have

proved effective in animal models including candidates against HIV, malaria and influenza[28]. DNA vaccines have been extensively evaluated in humans with a recent review identifying 72 Phase I, 20 Phase II and two Phase III human trials[29]. *Mycobacteria*, such as *Mycobacterium tuberculosis*, have received particular attention in this respect[30,31]. Finally, some common antigens might be present in a variety of different CF pathogens, and the development of a vaccine might have the potential for conferring cross-protection against several CF pathogens (see below).

Vaccines against opportunistic CF pathogens

Some infections[32,33] frequently associated with impaired respiratory function in patients with cystic fibrosis are the subject of vaccine development: for example infections with *P. aeruginosa*.

The development of a vaccine against *P. aeruginosa* has so far mobilized many research teams, even though no human trials have been conducted yet. Numerous vaccine attempts have been made against this pathogen that is generally considered to be the most targeted among pathogens infecting cystic fibrosis patients, and in order to obtain a state of the art overview in this domain, you would do well to explore the following recent reviews[34-37]. Major target antigens include the O-glycosylated lipopolysaccharide, cell-surface alginate, flagella, components of the Type III secretion system and outer membrane proteins[38]. The FliC flagellin protein widely considered as a virulence factor, has pro-inflammatory activity on respiratory epithelial cells. Flagellin has been one of the major targets for vaccines and therapeutic development expecially for CF patients. For example, as early as 1995, *P. aeruginosa* flagella were developed as a vaccine against *P. aeruginosa* and a Phase I study demonstrated that intramuscular immunization in healthy human adults results in high and long-lasting serum IgG flagella antibody titers and IgG, IgA and secretory IgA isotypes in the secretory immune system[39]. Then, in a phase III study, an immunization with a bivalent

vaccine for flagella subtypes significantly lowered the risk for initial *P. aeruginosa* infection in CF patients[40]. Other proteins have been used in human trials: OprF and OprI that are outer membrane proteins are able to induce a specific antibody response in the lung after nasal and oral vaccinations, and are as such promising candidates for the development of anti-pseudomonas immunization[41]. Furthermore, a fusion protein of the *P. aeruginosa* OprF fragment, OprI, and FliC promoted the clearance of *P. aeruginosa* in a pulmonary challenge model[42]. Another study which also tested these two outer membrane proteins as mucosal vaccines, lead to the development of airway immunogenicity against the pathogen with superior efficacy compared to systemic vaccination[43]. We can add to this, as more recently, among the latest antigens and strategies tested, an assay using the conserved surface exopolysaccharide alginate, a virulence factor produced by mucoid strains, has been tried in mice[44] and conferred protection after intranasal challenge. It was also efficacious as a therapeutic vaccine. Previously, an assay was performed in humans with O-polysaccharide conjugated to toxin A and vaccinated children developed less chronic *Pseudomonas* lung infections than non-vaccinated children[45,46]. An element of alginate (polymannuronic acid) has also been conjugated to flagellin leading to protective efficacy in a mouse lung infection model[47]. Some *P. aeruginosa* antigens conjugated to bovine serum albumin have also been tested in mice[48]. However, despite more than 50 years of research efforts, a licensed vaccine against *P. aeruginosa* is still a long way off from being available for CF patients.

With reference to the *Burkholderia cepacia* complex (Bcc), several virulence factors associated with human infection were tested for their potential as vaccine candidates[49]. Such an approach was undertaken by a group that sought immunoreactive proteins expressed by both *Burkholderia cenocepacia* and *Burkholderia multivorans*[50]. A recent review summarizes in detail all vaccination experiments that have been undertaken against Bcc[51]. As a recent example, the flagellar protein FliC from *Burkholderia pseudomallei* and considered as a

virulence factor has been shown to confer protection in mice[52]. This study shows that the epitopes of interest in *B. pseudomallei* FliC cross-react with orthologous *B. multivorans* and *B. cenocepacia* FliC sequences suggesting protection can be conferred against members of the Bcc. Other proteins like OMPs from different *Burkolderia* species are also able to generate protection in mice[53,54].

Vaccination in the context of mycobacteria.

The most widely used global vaccine is the "Bacille Calmette et Guérin" (BCG) strain of *Mycobacterium bovis* used in the fight against tuberculosis, a disease caused by *Mycobacterium tuberculosis*, which presently kills 1.3 million individuals around the world every year[55]. This technique harnessed the historically defined strategy developed by Jenner[56], by using the antigenic repertoire of a non-pathogenic strain for human, in order to confer protection against the human pathogen. Despite its widespread use, BCG is still a controversial vaccine, and its deficiencies have lead to the development of new research axes, using either purified compounds or a DNA vaccines in a quest to improve anti-mycobacterial vaccines. Several mycobacterial proteins (Ag85A/B, 65-kDa heat shock protein, hsp65, 36-kDa proline-rich antigen, MPB83, MPB70, CFP-10 and ESAT-6) have been evaluated as DNA vaccines in experimental models[57-63]. By virtue of its strong capacity to induce CD4+-mediated Th1 and CD8+-mediated cytotoxic T-lymphocyte responses[25], DNA vaccine approaches are particularly attractive for their preventive and therapeutic activity against intracellular pathogens such as pathogenic mycobacteria[61]. The majority of these studies were conducted in the fields of human[30,31,57,60,61] and bovine tuberculosis[58,59]; and even in leprosy (immuno-dominant 35-kDa protein[64]). Few DNA vaccines have been developed which are related to pathogenic or opportunistic NTM, for example *M. avium* (35-kDa protein[65]; p85A-

EGFP, p65K-EGFP[66]); *Mycobacterium ulcerans*[67] or *Mycobacterium marinum*[62,63]. None were developed in the context of tackling infections in inherited diseases such as CF.

While the presence of NTM is demonstrated in 7 to 13% of CF patients[8], infections by *M. tuberculosis* are rare in CF patients[68,69]. BCG remains a currently recommended vaccine in children at risk of exposure to TB; and as a consequence of partial efficacy against NTM, continues to be recommended in CF children. The two mycobacteria that together are responsible for the majority of infections in cystic fibrosis patients are *M. avium* and *M. abscessus*, a slow and a rapid growing mycobacteria (SGM and RGM), respectively, and to our knowledge, no vaccine approach against these bacteria has been considered in the context of CF. As mentioned above, development of vaccine targets can benefit from knowledge derived from genome sequence comparison[25]. Genome sequence comparison between the *M. abscessus* genome and *Mycobacterium chelonae*, *Mycobacterium smegmatis* genomes (two rapid growing NTM, which are less or non-pathogenic respectively), allowed the unraveling of several key virulence factors[19]. As described in each genome sequences, several of these virulence factors were acquired by horizontal gene transfer, from non-fermenting Gram negative bacteria such as those found in CF patient lungs: *P. aeruginosa* and Bcc[19]. Among others we characterized MAB_0555, a phospholipase C (PLC) with the highest homology with PLC-N from *P. aeruginosa*[70]. We have demonstrated the impact of MAB_0555 PLC in the virulence of *M. abscessus* in mice[70], when pre-cultivated on amoeba. We have also shown that the recombinant protein or the plasmid encoding PLC conferred protection, after an aerosol or IV challenge (Figure 1), in Δ508 or CF mice[71] only[26]. The two formulations (Figure 1) gave quite similar results, namely a diminution of bacterial load in lungs three weeks after a *M. abscessus* aerosol challenge[26]. PLC are also present in other CF pathogens like *P. aeruginosa* and the immune cross reactivity between *M. abscessus* PLC and *P. aeruginosa* PLC could lead to a vaccine protective against both mycobacterial and Gram negative

infection in CF patients, as we were able to show the recognition of the MAB_0555 PLC by sera from CF patients only infected by *P. aeruginosa*. This approach is currently underway in our laboratory with other target designs recently unraveled[72].

Conclusion

Vaccination is effective in preventing infections and we advocate their use in patients with CF, specifically for the prevention of respiratory infections. In addition to the traditional vaccination schedules,[73] evaluation studies to demonstrate the immunological and clinical efficacy of novel vaccines against multi-resistant bacteria remain necessary in this particular patient population.

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