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Stratégies vaccinales contre les bactéries pathogènes chez les patients atteints de mucoviscidose

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Résumé

De nombreux pathogènes associés à la mucoviscidose, tels que les bactéries du complexe *Burkholderia cepacia*, *Pseudomonas aeruginosa* ou *Mycobacterium abscessus* posent des problèmes thérapeutiques complexes en raison de leur multirésistance intrinsèque aux antibiotiques. De plus, aucun vaccin n'est actuellement disponible contre ces pathogènes. Les approches vaccinales représentent donc une arme clé pour combattre ces bactéries multirésistantes dans un certain nombre de cas cliniques, dont celui de la mucoviscidose. Différentes stratégies peuvent être envisagées pour développer ces vaccins. Certains facteurs de virulence similaires sont exprimés au cours de l'infection par différents pathogènes et pourraient ainsi être utilisés comme antigène pour évaluer une protection croisée. De nombreux essais sont en cours pour tenter de générer une prophylaxie dans le cadre de la mucoviscidose.

Abstract

A large number of cystic fibrosis pathogens such as bacteria of the *Burkholderia cepacia* complex, *Pseudomonas aeruginosa*, or *Mycobacterium abscessus* are associated with complex therapeutic problems due to their inherent resistance to antibiotics. No vaccine is currently available against those pathogens. Vaccines are therefore crucial to combat these multidrug-resistant bacteria in specific clinical situations including cystic fibrosis. Various strategies may be considered to develop these vaccines. Similar virulence factors are expressed during the infection with various pathogens; they could thus be used as antigen to assess cross-protection. Many clinical trials are currently being conducted to try and develop a prophylactic treatment for patients presenting with cystic fibrosis.

Cystic fibrosis (CF) is a Mendelian genetic disease caused by a series of mutations occurring in the coding gene for the CFTR protein, which acts as a chloride channel [1]. The absence or lack of efficacy of that protein is responsible for the increased mucus viscosity, especially in the lungs. Bacteria can thus more easily accumulate and adhere to mucins. Chronic inflammation [2] and early bacterial infection are both responsible for the subsequent deterioration of the lungs. Lung infections in CF patients are the most frequent and severe presentations of the disease. They account for more than 90% of deaths [3]. Bacteria, fungi, and viruses can infect the patient's respiratory system. Bacterial colonization occurs very early in the disease progression [4]. Patients first present with *Haemophilus influenzae* and *Staphylococcus aureus* colonization. Months or years later patients are colonized with *Pseudomonas aeruginosa*. *Burkholderia cepacia* is the fourth bacterium mainly responsible for CF patients' lung infection. The administration of an antibiotic treatment is the only effective strategy to combat the infection. However, bacteria become resistant to antibiotics when repeatedly used. A single bacterium can have many different strains and can easily mutate. Developing effective treatment strategies is therefore difficult.

Resistance to antibiotics is a major problem for CF patients. Multidrug-resistant bacteria such as *B. cepacia*, *P. aeruginosa*, or *Mycobacterium abscessus* lead to therapeutic difficulties and are responsible for fatal infections [5].

Guidelines focusing on prophylaxis must therefore be drawn and therapeutic strategies for respiratory tract infections must be developed. Such therapeutic strategies should be integrated into the overall disease management.

The *Burkholderia cepacia* complex (Bcc) consists of 18 species responsible for opportunistic infections that can be life-threatening in CF patients. *Burkholderia cenocepacia* and *Burkholderia multivorans* are most frequently identified. These environmental and biofilm-

forming intracellular bacteria are highly resistant to antibiotics. Bcc infections developing in CF patients are rarely eliminated once a patient is colonized. *Pseudomonas aeruginosa* is another environmental pathogen responsible for opportunistic infections. It is the most frequent bacterium isolated from CF patients. *P. aeruginosa* colonization and chronic infections affect up to 80% of CF adult patients [6]. This pathogen is responsible for chronic endobronchial infections and increases morbidity and mortality rates. *P. aeruginosa* is resistant to antibiotics. It is therefore a dangerous pathogen as once patients are colonized the pathogen is rarely, or even never, eliminated. *P. aeruginosa* colonization usually affects the lungs of CF patients. The bacterium forms a biofilm on the lungs and reduces the patient's immune response, thus contributing to the bacterium high level of resistance to antibiotics [7]. *Mycobacterium abscessus* is the most recently identified bacterium to be highly resistant to antibiotics. It is a rapidly-growing mycobacterium belonging to the *Mycobacterium abscessus* complex [8]. *Mycobacterium abscessus* is responsible for a wide variety of human diseases, especially in CF patients [9, 10]. Person-to-person transmission has recently been reported in CF patients [11, 12]. *M. abscessus* is associated with major therapeutic difficulties because of its natural resistance to most antibiotics [13, 14]. Severe and even fatal infections have already been reported in CF patients due to the lack of therapeutic strategies [15]. Several countries consider that patients presenting with a *M. abscessus* infection cannot be eligible for lung transplant [16]. CF patients presenting with such infection are therefore left with no therapeutic option.

Acute or chronic bronchial infections and superinfections progressively deteriorate the patient's respiratory function. They are treated with antibiotics in light of the bacteriological examination results. Sputum culture (sputum cytobacteriological examination) or blood samples (blood cultures) allows for identifying the involved bacterium, evaluating the extent of the colonization, and determining the most effective antibiotics. The most frequently

observed bacteria (*S. aureus*, *P. aeruginosa*, and *B. cepacia*) are rapidly resistant to antibiotics. The most effective doses are still unclear but they are usually higher than the ones recommended in the agents' marketing authorization. The administration of two intravenous antibiotics is, for instance, often combined with an inhaled maintenance antibiotic treatment. Inhaled antibiotics must be administered after chest physical therapy (CPT) and after having administered beta-2-agonists and rhDNase.

For *S. aureus* infections, a primary prophylactic treatment is not recommended for infants and children. For methicillin-susceptible *S. aureus* exacerbations, the recommendation is to first administer oral beta-lactams as first-line treatments with or without fusidic acid. Treatment duration is at least 14 days. For methicillin-resistant *S. aureus* exacerbations, it is recommended to administer a combination of pristinamycin and rifampicin. There is currently no recommendation for secondary antibiotic prophylaxis (or maintenance treatment) as there is no consensus on that matter.

For *P. aeruginosa* infection, it is recommended to first administer two intravenous bactericidal antibiotics (14-21 days) to patients presenting with a primary colonization (beta-lactams + aminoglycoside). Inhaled colistin may then be prescribed for 3 to 6 months. Exacerbations of patients presenting with chronic infection should be treated with a combination treatment to prevent the emergence of resistant strains: beta-lactam and tobramycin for at least 14 days. For a multidrug-resistant strain, a combined treatment with three antibiotics should be administered including oral ciprofloxacin or intravenous colistin. Although there is currently no guideline recommending such treatment, a maintenance antibiotic treatment (inhaled) administered for 28 days or IV treatments administered every three months, preferably at home, may be considered.

A significant association between previous intravenous antibiotic treatments and *M. abscessus* isolation in the lungs of CF patients has recently been reported. Such association highlights

the role of a broad spectrum antibiotic treatment in the occurrence of *M. abscessus* infection [17].

Vaccination strategies

Pathogens can be divided into two groups: vaccine-preventable pathogens and non-vaccine-preventable pathogens. There is currently no human vaccine against most antibiotic-resistant pathogens previously mentioned. It would thus be interesting to develop a prophylactic vaccination strategy to improve the prevention of those infections. Reverse-vaccinology is interesting as it would help target antigens associated with strong vaccine effectiveness. A better understanding of the regulation of bacterial gene expression helps in developing new strategies to combat such bacteria [18].

Various vaccination strategies can be considered once a potential target is identified: conventional vaccination using a recombinant protein or vaccination using a plasmid DNA encoding the antigen.

Various pathogens may contain highly similar antigens acting as virulence factors. Those antigens could be used to ensure a potential cross-protection. For instance, *M. abscessus* phospholipase C (PLC) could be a joint vaccine target against PLC-producing CF pathogens such as *P. aeruginosa*. This would be an interesting strategy as the serum of *P. aeruginosa*-infected patients contains antibodies that recognize *M. abscessus* recombinant PLC [19].

Conventional vaccination schedule

CF patients are advised to comply with the recommended vaccination schedule (diphtheria, tetanus, poliomyelitis, acellular pertussis, *Haemophilus influenzae* type B, *Streptococcus pneumoniae,* hepatitis B, measles-mumps-rubella) as well as with hepatitis A and influenza vaccination.

Haemophilus influenzae often colonizes the respiratory tract of CF patients. The prevalence of non-typable *Haemophilus* strains is increasing [20]. The vaccination for *Haemophilus influenzae* type b has proved effective in preventing invasive diseases [21]. However, no study has so far been conducted to assess its benefits for CF patients.

Streptococcus pneumoniae is the fourth bacterium frequently isolated from respiratory samples of CF patients after *S. aureus*, *P. aeruginosa*, and *H. influenzae*. However, invasive infections due to *Streptococcus pneumoniae* are rare in those patients [22]. The population of patients infected by *Streptococcus pneumoniae* has changed since the introduction of the pneumococcal conjugate vaccines (PCV7 and PCV13) [23]. Although CF patients rarely contract pneumococcal disease, the United Kingdom and United States advise CF patients to get the pneumococcal vaccine [24]. No study has so far been published on the effectiveness of such vaccine in that population of patients. However, Browning *et al.* [25] observed that a subgroup of CF children poorly responded to a pneumococcal polysaccharide vaccine (Pneumovax II). This may be due to disease severity.

Influenza-related respiratory tract infections in CF patients deteriorate lung function and contribute to disease progression. Yearly influenza vaccination is strongly recommended for CF patients aged above 6 months. However, there is no evidence based on randomized studies on the benefit of such vaccine for those patients [26]. Recent data does not suggest that influenza develops more frequently in CF patients than in healthy individuals [27]. The

influenza vaccine is however recommended for CF patients. Launay *et al.* [28] observed that CF patients presenting with malnutrition and receiving the non-adjuvanted vaccine had a lower immune response to the pandemic influenza vaccine. Their data also suggests that CF patients had an inadequate immune response to the influenza vaccine. One may thus wonder whether a different vaccination strategy is necessary.

All other vaccinations are based on a specific vaccination schedule and are not subject to specific recommendations for CF patients.

Vaccines currently being developed

Vaccine development is currently focusing on some infections frequently associated [29,30] with respiratory failure in CF patients: *Pseudomonas aeruginosa*, *Staphylococcus aureus* infections, or respiratory syncytial virus (RSV) infections.
Many researchers are focusing on developing a vaccine against *P. aeruginosa* even though no clinical trial has so far been successful in supporting the benefits of a specific vaccine. Many vaccines have been tested against *P. aeruginosa*. The results of recent studies are useful to get an overview of the current state of vaccine development for CF patients [31-34]. Major target antigens include the lipopolysaccharide *O*-polysaccharides, cell-surface alginate, flagella, components of the Type III secretion system, and outer membrane proteins [35]. The flagellin protein (FliC) is thought to be a virulence factor and is a major component of flagella. The protein induces inflammation on respiratory epithelial cells. Just like in *Burkholderia cepacia*, flagellin was one of the main targets for vaccine trials and especially for CF patients. In 1995, *P. aeruginosa* flagella were already developed as a *P. aeruginosa* vaccine. Results of phase I clinical trials showed that intramuscular immunization of healthy adults resulted in high and long-lasting serum IgG antibody titers against flagella antigens and also elicited specific

antibodies to flagella of the IgG, IgA, and secretory IgA isotypes in the secretory immune system of CF patients [36]. The results of a phase III trial then revealed that the administration of a bivalent *P. aeruginosa* flagella vaccine, exhibiting flagella subtypes, significantly decreased the risk of *P. aeruginosa* initial infection in CF patients [37]. Other proteins have been used in human clinical trials such as the OprF and OprI proteins. They are both outer membrane proteins able to induce a specific antibody response in the lungs after nasal or oral vaccination. Both of these proteins are good candidates for a *P. aeruginosa* vaccine [38]. A fusion protein from a part of *P. aeruginosa* OprF, OprI, and FliC helped in *P. aeruginosa* clearance in a pulmonary challenge model [39]. Another clinical trial evaluated the role of these two outer membrane proteins in a mucosal vaccine. Its results indicated respiratory tract immunogenicity against the pathogen with better effectiveness than systemic vaccination [40]. One of the most recent clinical trials evaluating antigens and immunization strategies used a virulence factor produced by mucoid strains known as surface exopolysaccharide alginate [41]. The clinical trial was conducted with mice: vaccinated mice were protected when challenged intranasally with *P. aeruginosa*. The virulence factor therefore seems to be effective as a therapeutic vaccine. A human trial had previously been conducted with O-polysaccharide-toxin A conjugate *P. aeruginosa* vaccine. The results indicated that immunized children presented with fewer chronic *P. aeruginosa* lung infection than non-immunized patients [42,43]. The authors of another study conjugated a part of the alginate (polymannuronic acid) with flagellin and obtained a high level of protection in lung-infected mice [44]. Some *P. aeruginosa* antigens have also been conjugated with bovine serum albumin and tested on mice [45]. A human vaccine targeting *P. aeruginosa* is, however, still not available despite more than 50 years of research.

With regard to *Burkholderia cepacia* complex, various virulence factors associated with the occurrence of the infection in humans have been tested as potential vaccines [46]. That same

strategy had already been considered by a group of researchers looking for immunoreactive proteins expressed by both *Burkholderia cenocepacia* and *Burkholderia multivorans* [47]. The authors of a recent literature review took a detailed inventory of vaccination studies performed in relation to the *Burkoldheria* genus [48]. Within the related species *Burkholderia pseudomallei*, the flagellar protein (FliC) can be considered as a virulence factor and has been used as an antigen in a recent trial. The protein had a protective effect on mice [49]. The results of the study also revealed that *B. pseudomallei* FliC epitopes of interest cross-reacted with orthologous FliC sequences from *Burkholderia multivorans* and *Burkholderia cenocepacia*. Those epitopes could thus be integrated into vaccination programs. Other proteins such as outer membrane proteins from various *Burkolderia* species can also induce protection in mice models [50,51]. Although research is still focusing on finding the adequate target for a prophylactic treatment, vaccine development research has not yet managed to go beyond human clinical trials [48].

Staphylococcus aureus is usually the first bacterium isolated from the respiratory secretions of CF patients [52] and its prevalence is currently increasing [53]. The affinity of *Staphylococcus aureus* for cystic fibrosis mucus, mucociliary abnormalities, and other unknown factors contributes to persistent colonization with this bacterium. It causes progressive pulmonary damage and may facilitate *Pseudomonas* infections [52]. Initial effort to develop a *Staphylococcus aureus* vaccine was based on conventional immunologic mechanism: a humoral-based vaccine [54]. The first attempts were however unsuccessful in reducing the risk of contracting invasive *Staphylococcus aureus* infections [55,56]. A new immunologic strategy is therefore needed to develop a *Staphylococcus aureus* vaccine. This may be done by inducing memory T cells which are capable of increasing the rapidity and

strength of phagocyte recruitment to infection sites, thus facilitating clearance of the bacterium from tissues [54].

One may also mention the role of the RSV. RSV infections can indeed deteriorate the respiratory function of children presenting with CF. RSV might also be involved in the initial respiratory tract infection by *P. aeruginosa* [57]. Vaccines have been tested [58] but there is once again no available vaccine yet.

Vaccination and mycobacteria: benefits of the BCG vaccine

Nontuberculous mycobacteria are identified in 7 to 13% of respiratory samples isolated from CF patients [9]. However, *M. tuberculosis* infections are not common in industrialized countries [59,60]. The BCG vaccine is currently recommended for children at risk of tuberculosis exposure. The vaccine is effective on nontuberculous mycobacteria and should always be given to CF children.

Mycobacterium avium and *Mycobacterium abscessus* are mainly responsible for mycobacterium infections in CF patients. *Mycobacterium avium* is a slow-growing mycobacterium (SGM) while *Mycobacterium abscessus* is a rapidly-growing one (RGM). *Mycobacterium abscessus* infections are more severe and the mycobacterium is resistant to antibiotics. Vaccination strategies have so far not been studied for CF patients presenting with such infections. Comparing whole-genome sequencing may help in the research for target vaccines [61]. A reverse-vaccinology strategy using whole-genome sequencing was considered to develop a vaccine against that bacterium [13]. A first antigen was selected on the basis of its pathogenic role: MAB_0555, encoding PLC [62]. Further antigens were then selected and are currently being tested. An *in silico* subtractive genome analysis allowed for identifying MAB_0555 in the genome of *M. abscessus* and for confirming its absence from

both *M. smegmatis* and *M. chelonae* genomes, which are two RGM respectively less-pathogenic or non-pathogenic. *M. abscessus* is the only RGM to be a major respiratory pathogen in CF patients. CF mice vaccination (ΔF508) [63] against this antigen was performed either using the recombinant protein or a coding plasmid for that antigen to perform a DNA vaccination [19]. Similar results were observed with both formulations: a decreased bacterial load in the lungs after three weeks of aerosolized *M. abscessus* challenge [19]. PLC are also present in other CF pathogens such as *P. aeruginosa*. Crossed-immune reaction between *M. abscessus* PLC and that of *P. aeruginosa* could help develop a protective vaccine against both mycobacterium infections and Gram-negative infections in CF patients.

Conclusion

Vaccines are effective in preventing infections. Vaccination is strongly recommended for CF patients, especially to prevent respiratory tract infections. In addition to conventional vaccinations, evaluation studies aiming to show the immune and clinical effectiveness of new vaccines targeted against multidrug-resistant bacteria are still needed for CF patients.

Conflict of interest
The authors report no conflict of interest

Authors' contribution
All authors contributed to writing the article.

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