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Factors associated with humoral immune response to pandemic A/H1N1(v) 2009 influenza vaccine in cystic fibrosis

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ABSTRACT

Influenza vaccination is recommended in cystic fibrosis patients. The objective of this study was to assess the immunogenicity of vaccination against 2009 pandemic A/H1N1 influenza and to study the factors associated with the immune response in patients with cystic fibrosis.

122 patients with cystic fibrosis were enrolled in a prospective study and received 1 dose of 2009/H1N1v adjuvanted vaccine, or for children <2 years and lung-transplanted patients, two doses of non-adjuvanted 2009/H1N1v vaccine administered 21 days apart. Hemagglutination inhibition antibodies were assessed before and 21 days after vaccination and at least 6 months after vaccination.

After vaccination, 85% of the patients had an influenza antibody titer ≥1:40 and 69% seroconverted. 13% of the transplanted patients seroconverted compared with 72% of the non-transplanted patients. In this latter group, non-adjuvanted vaccine and low body mass index were independently associated with lower response to vaccination. 86% of the non-transplanted patients with normal BMI and receiving adjuvanted vaccine seroconverted. Persistence of seroprotection 10 months after vaccination was found in 50% of the patients.

In patients with cystic fibrosis, malnutrition and receipt of non-adjuvanted vaccine were associated with lower immune response to pandemic influenza vaccination. Our data also suggest a potential defect in the immune response to influenza vaccination of patients with cystic fibrosis and raise the question of whether a different immunization strategy is needed.

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1. Introduction

Patients with cystic fibrosis (CF) are at increased risk of influenza-associated exacerbation and respiratory deterioration [1]. Moreover, there is evidence that respiratory viruses, including influenza, may promote new colonization by *Pseudomonas aeruginosa* and chronic infection [2,3]. Annual vaccination against influenza is therefore strongly recommended in CF patients to prevent progression of lung disease [4].

Paradoxically, only a few studies have evaluated influenza vaccinations in CF patients [5–9] so that scientific evidence about the

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immunogenicity and clinical effectiveness of immunization against influenza in these patients is scarce [10]. Moreover, the impact of CF-related conditions that may limit the vaccine response, such as solid-organ transplants, diabetes, hepatic cirrhosis and malnutrition have not been evaluated.

In 2009, pandemic influenza A/H1N1v prompted new discussions about influenza vaccination in CF. Patients with CF were designated as a top priority group to receive the 2009 influenza A/H1N1 vaccine because of the potentially severe consequences of this viral infection on CF lung disease [11]. However, some patients with CF and their physicians expressed concern about the efficacy of the influenza vaccine as well as fears about the vaccination's side effects [12].

This controversy led us to assess the immunogenicity of vaccination against pandemic influenza A/H1N1 and to study the factors potentially associated with lower immune response. We present here the results of a prospective study conducted in 122 patients with CF that aimed to investigate seroprotection/seroconversion rate to vaccination against 2009 pandemic A/H1N1 influenza.

2. Methods

2.1. Study design

Mucoflu (Clinical Trials.gov registration number: NCT-01499914) was a prospective study initiated by INSERM (Institut National de la Santé et de la Recherche Médicale) and APHP (Assistance Publique des Hôpitaux de Paris) to study pandemic flu vaccination in CF patients and conducted in the CF referral centers and lung transplant centers of the Paris metropolitan area.

All CF patients older than 6 months and enrolled in MucoFlu were asked to participate in the vaccination sub-study. Exclusion criteria for enrolment in this sub-study were: (1) allergy to eggs or other components of the vaccine or a history of severe reaction after influenza vaccination, (2) virologically documented infection with 2009 A/H1N1 influenza in the past 6 months, (3) a febrile episode in the week before vaccination and (4) another vaccination in the 3 weeks before study entry or planned in the next month.

Written informed consent was obtained from each patient before enrolment and from the parents of the patients younger than 18 years. The protocol was approved by the Ile de France III Ethics Committee (Paris, France).

2.2. Intervention

Patients were enrolled in the study and vaccinated by the nursing staff in their referral CF centers against 2009 pandemic A/H1N1 influenza from November 2009 to January 2010 according to the French national recommendations. A single intramuscular injection in the non-dominant arm of AS03-adjuvanted vaccine containing 3.75 μg hemagglutinin (HA) (Pandemrix®, GlaxoSmithKline) was recommended from 2 years of age [13]. Children younger than 24 months or patients who had had lung transplants had 2 doses of the non-adjuvanted vaccine containing 15 μg HA (Panenza®, Sanofi-Pasteur) 21 days apart: 2 half doses for the children and 2 doses for the transplant patients [13]. Moreover, considering the controversy around the limited experience of adjuvant [12], some patients or families specifically asked for the non-adjuvanted formulation, according to a specific age-related schedule (i.e.; 2 half doses for the children below 3 years of age, 2 doses between 3 and 9 years of age, and 1 dose for the older ones) [13].

2.3. Follow-up

Blood samples were collected for assessment of hemagglutination inhibition (HI): (1) at day 0 before the pandemic A/H1N1 influenza vaccination, (2) at day 21 after vaccination for patients who had received a single dose or days 21 and 42 after vaccination for those who had received two doses, and (3) between 6 and 10 months after vaccination, during one of their scheduled appointments with the CF Center. All serum specimens were stored at −80 °C and assayed simultaneously.

All the patients were reviewed clinically at least every 3 months until the next influenza season (February 2011). They were instructed to contact the CF Center for any Influenza-Like Illness, whose symptoms were clearly defined in a leaflet given to the patients at the beginning of the epidemics. Cases of influenza A/H1N1 occurring at least 14 days after vaccination and confirmed by positive A/H1N1 PCR either in sputum or nasal secretion were reported as vaccine failures. The number of respiratory exacerbations requiring antibiotics, new bacterial identification, and forced expiratory volume in 1 s (FEV1), expressed as percentage of the predicted value for sex, age and height according normative data, at study entry and at the 1-year follow-up, were compared in each group.

The primary study endpoint was the humoral immune response (seroconversion, seroprotection). Secondary endpoints were safety and influenza infections. Long term follow-up of clinical conditions and long term HI antibody titers were only exploratory endpoints.

2.4. Hemagglutination inhibition antibodies against influenza

Immunological assays were performed on a blinded basis in a centralized laboratory (Virology Laboratory, Cochin Hospital, Paris, France). The titer of antibodies against the vaccine strain was measured by hemagglutination inhibition (HI), modified from the WHO Collaborating Center for Influenza Center for Diseases Control, Atlanta, USA, protocol [14]. Serum samples were treated by enzymatic treatment and heated to destroy non-specific inhibitors. Samples were then tested in serial 2-fold dilutions starting at 1:10, with all sera from a single patient tested on the same plate. Hemagglutination was performed in a microtiter plate with human O rhesus-negative erythrocytes and 4 units of A/California/7/2009 (H1N1v) vaccine used as antigen. Baseline sera were also tested against A/Brisbane/59/2007 antigen, a component of the seasonal trivalent vaccine in 2009/2010. The serum titer was the highest dilution that completely inhibited hemagglutination. Negative samples were assigned a titer of 1:5. The geometric mean of HI antibody titers at each time point were used for the analyses. This allowed two different specific evaluations against pandemic (H1N1v) and seasonal (Brisbane) A influenza [15].

2.5. Adverse event assessment

Local and general reactions were collected for 30 min after vaccination. Patients were then provided with diary cards to record the occurrence and severity of specific local reactions at the injection site and specific general reactions for 21 days after each vaccine injection. Long term adverse events were also reported prospectively for 1 year as part of the Mucoflu study.

2.6. Statistical analysis

Immunogenicity was analyzed by the standard HI endpoints (with their 95% confidence intervals) used by regulatory authorities to evaluate influenza vaccines [14]. In this descriptive study, we planned to include 100 patients so that seroprotection could be estimated with 10% precision. Seroconversion was defined by a ≥4-fold increase in HI antibody titer from pre- to post-vaccination samples, and seroprotection by achievement of a post-vaccination HI titer of ≥1:40 or more. HI titers were used to calculate seroconversion rates (% of patients achieving seroconversion), seroprotection

Table 1
Participant characteristics.

	Adjuvanted vaccine group ($n=91$)	Non-adjuvanted vaccine non transplanted group ($n=16$)	Non-adjuvanted vaccine lung transplant recipients group ($n=15$)
Age (years)/mean (SD)	21.8 (9.6)	18.0 (16.4)	25 (8.5)
Sex (male)/*n* (%)	51 (56)	3 (19)	7 (47)
BMI Z-score/mean (SD)	−0.35 (1.10)	−0.17 (1.05)	−1.41 (1.23)
FEV1 (% predicted)/mean (SD)	64 (25)	62 (33)	75 (26)
P.aeruginosa colonization/n (%)	43 (47)	8 (50)	6 (40)
S.aureus colonization/*n* (%)	32 (35)	4 (25)	2 (13)
Pancreatic insufficiency/*n* (%)	74 (81)	13 (81)	13 (87)
Diabetes/*n* (%)	17 (19)	2 (12)	8 (53)
Hepatic cirrhosis/*n* (%)	2 (2)	0	0

BMI: Body Mass Index; FEV_1: Forced Expiratory Volume in one second expressed as % of the predicted value for sex, age and height; colonization is defined by at least 3 identifications of the microorganism in sputum during 3 months.

rates (% of patients with HI titers of 1:40 or greater) and the seroconversion factor (geometric mean fold rise in antibodies) for each immunization group. Exact 95% binomial confidence intervals were used for proportions. For the geometric mean titer (GMT) and the geometric mean fold rise, means and 95% confidence intervals were calculated for the $\log_{10}$ transformed titers and then transformed back to the original units by exponentiation. Age was analyzed as a 2-class variable (<9 years as the reference class). BMI Z-scores were divided in quartiles, with the lowest quartile the reference class. As patients with lung transplants were always vaccinated with the non-adjuvanted vaccine, we reported results in three subgroups: lung-transplanted patients, non-transplanted patients vaccinated with the non-adjuvanted vaccine and non-transplanted patients vaccinated with the adjuvanted vaccine. Quantitative variables were compared using the Wilcoxon test. Qualitative variables were compared using Fisher's exact test. A *p*-value less than 0.05 defined statistical significance.

Logistic regression was used to analyze risk factors for a lower seroconversion rate in non-transplanted patients, such as low BMI, diabetes and hepatic cirrhosis. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were calculated. All analyses were conducted with the statistical software R 2.13.

3. Results

3.1. Study population

One hundred and twenty-two patients were enrolled from November 13, 2009 to February 1, 2010, 91 of whom received the adjuvanted vaccine. Of the 31 who had the non-adjuvanted vaccine, 18 followed the French national recommendations, i.e.; 15 had undergone lung transplantation and 3 were younger than 2 years. Thirteen other patients, including 7 children above 2 years of age and 6 adults from 22 to 49 years, also asked to be immunized with the non-adjuvanted vaccine. Patient characteristics are described in Table 1.

All the patients had been vaccinated with non-adjuvanted seasonal influenza vaccine in October 2009. At time of vaccination against pandemic 2009 A/H1N1 flu, 35 patients (29%) had no seroprotective titers against seasonal A/H1N1 Brisbane strain (HI titers < 1:40). They were older than the patients who were seroprotected (mean age 24 years versus 20 years ($p<0.02$)). The two groups did not differ by transplantation status, nutritional status, or diabetes.

3.2. Immune response to 2009 pandemicA/H1N1 influenza vaccination

Before pandemic influenza vaccination, 21 patients (17%) had HI antibodies against 2009 A/H1N1v of 1:40 or greater, whereas they did not report previous typical influenza symptoms. Three weeks after vaccination, 104 patients (85%) had HI antibodies against A/H1N1v of 1:40 or greater (Table 2); 84 (69%) seroconverted, for a geometric mean fold rise of 7 (95% CI, 5.6–8.9) (GMT ratio). Among the 38 patients who did not seroconvert, 5 did not achieve seroprotection for seasonal flu either, although they had been vaccinated against it from 2 to 4 months before the vaccination against pandemic A/H1N1. Only 13% of the transplanted patients seroconverted compared with 38% of the non-transplanted patients vaccinated with the non-adjuvanted vaccine and 84% of the non-transplanted patients vaccinated with the adjuvanted vaccine ($p<0.0001$) (Table 2).

In the multivariate analysis conducted in the non-transplanted patients, the non-adjuvanted vaccine and a low BMI Z-score were associated with a lower seroconversion rate. Specifically, 38% of the patients receiving the non-adjuvanted vaccine seroconverted, compared with 84% of those receiving the adjuvanted vaccine. Similarly, a low BMI Z-score (<−1.2, i.e.; in the lowest quartile of BMI Z-score in the whole population) was associated with a lower seroconversion rate (53% versus 75% among the patients in the higher quartiles, $p=0.048$). Age below 9 years, diabetes and seroprotection against A/H1N1 Brisbane strain did not decrease the response to pandemic H1N1 vaccine (Table 3). Interestingly, 9/66 (14%) patients with neither of these risk factors (i.e., vaccinated with the adjuvanted vaccine and BMI Z-score >−1.2) failed to seroconvert.

All the transplanted patients were treated with tracrolimus and corticosteroids. One non-transplanted patient was treated by systemic corticosteroids for allergic broncho-pulmonary aspergillosis and experienced seroconversion after vaccination with the adjuvanted vaccine.

None of the patients enrolled had been lost to follow-up but only 75 were sampled for serologic evaluation between 6 and 10 months in the long term follow up. Thirty-eight (50%) maintained HI titers of 1:40 or greater (54% were vaccinated with the adjuvanted vaccine and 44% were vaccinated with the non-adjuvanted vaccine) (NS).

The proportion of patients with persistent seroprotection did not differ significantly between the transplanted patients and the non-transplanted patients vaccinated with either the adjuvanted or the non-adjuvanted vaccine (respectively, 60.5%, 68% and 60%) (see supplemental material). There were no demographic differences between the patients who underwent the long term serological sampling and those who dropped out.

3.3. Clinical follow-up during 1 year after vaccination against pandemic A/H1N1v influenza

During the 12 months after vaccination, no case of pandemic A/H1N1v influenza was reported in either the patients who were seroprotected after the vaccination or those who were not. At 1 year after vaccination, there were no significant differences for FEV1, sputum colonization and number of antibiotic courses between the patients who achieved seroprotection versus those who did not (Table 4).

3.4. Safety

Table 5 summarizes the adverse events reported following vaccination. No severe adverse events were reported. 55 patients

Table 2
Immune response to vaccination against pandemic influenza A/H1N1v virus.

	Adjuvanted vaccine group ($n=91$)	Non-adjuvanted vaccine Non-transplanted group ($n=16$)	Non-adjuvanted vaccine lung transplant recipients group ($n=15$)	All ($n=122$)
Pre-vaccination (day 0)				
Geometric mean titer (95% CI)	15 (12–18)	14 (9–24)	20 (12–35)	15 (13–18)
% of patients with HI titers ≥ 1:40 (95% CI)	13 (8–22)	25 (10–49)	33 (15–58)	17 (12–25)
Post-vaccination				
Geometric mean titer (95% CI)	136 (109–169)	64 (33–131)	38 (19–79)	107 (105–109)
Seroprotection rate[a] (95% CI)	95 (88–98)	69 (44–86)	47 (25–70)	85 (78–90)
Seroconversion rate[b] (95% CI)	84 (75–90)	38 (18–61)	13 (4–38)	69 (60–76)
Seroconversion factor[c] (95% CI)	9.2 (7.2–11.7)	4.6 (2.5–8.8)	1.9 (1.3–3.0)	7 (5.6–8.9)

CI: confidence interval.
[a] Seroprotection rate: % of patients with HI titers ≥1:40.
[b] Seroconversion rate: % of patients with a ≥4-fold increase in hemagglutination inhibition (HI) antibody titer.
[c] Seroconversion factor: Geometric mean fold rise.

Table 3
Variables associated with low conversion rates to influenza A/H1N1 vaccine in non-transplanted patients.

	Univariate analysis		Multivariate analysis[a]	
Variables	OR for seroconversion (95% CI)	p	OR for seroconversion (95% CI)	p
Non-adjuvanted vaccine (versus adjuvanted)	0.12 (0.04–0.4)	<0.001	0.15 (0.04–0.4)	0.009
BMI Z-score <−1.2	0.4 (0.1–1.2)	0.13	0.3 (0.1–1.0)	0.048
Diabetes	0.9 (0.2–3.8)	0.9	NA	–
Age <9 years	0.4 (0.1–1.4)	0.17	0.5 (0.06–5.0)	NS
HI titers against influenza A/Brisbane ≥1:40	0.7 (0.2–1.9)	0.45	NA	–

NA: Not applicable. Only the factors with $p<0.2$ in the univariate analysis are included in the multivariate analysis.
[a] Adjusted for age.

Table 4
Clinical follow-up of study participants at 1 year after vaccination according to seroprotection status (HI antibodies titers ≥1:40) against A/H1N1 influenza.

Outcome	Not seroprotected group ($n=37$)	Seroprotected group ($n=38$)	p
Death	0	0	NS
FEV1 (% predicted)/mean (SD)			NS
At enrolment	65%(27)	63%(24)	
At 1-year follow-up	66%(24)	64%(23)	
Staphylococcus aureus (% of patients colonized)			NS
At enrolment	52%	64%	
At 1-year follow-up	48%	61%	
Pseudomonas aeruginosa (% of patients colonized)			NS
At enrollment	68%	58%	
At 1-year follow-up	56%	62%	
No. of oral antibiotic courses (mean (SD))			NS
1 year before enrolment	2.5(2.3)	2.7(1.9)	
At 1-year follow-up	2.3(1.7)	3(2.1)	
No. of IV antibiotic courses (mean (SD))			NS
1 year before enrolment	1.8(2.4)	1.4(1.6)	
At 1-year follow-up	1.4(1.7)	2(2.4)	

Table 5
Local and general adverse events reported during 21 days after A/H1N1 influenza vaccination according to the type of vaccine.

	Total	Adjuvanted vaccine	Non-adjuvanted vaccine
Any local reaction			
Pain	25	15	10
Eythema	6	4	2
Edema	3	1	2
Others	8	4	4
Any systemic reaction			
Asthenia	1	0	1
Headache	3	2	1
Myalgia	6	1	5
Fever	5	3	2
Pulmonary exacerbation	7	4	3
Nausea, vomiting	4	3	1
Transient polyarthritis	3	3	0
Diarrhea	1	1	0
Cutaneous eruption	1	1	0

reported 72 mild adverse events related to the vaccination. Pain at the injection site was noted by 25 (21%) patients, and 14 (11%) reported moderate symptoms of myalgia, fever, headache or vomiting within 48 h after vaccination.

All the adverse events were mild or moderate. Myalgia was more predominant among the patients vaccinated with the non-adjuvanted vaccine and polyarthritis among those vaccinated with the adjuvanted vaccine. However, as a whole, there was no difference in the proportion of vaccines experiencing those symptoms according to the type of vaccine. Pulmonary exacerbation within 1 week after vaccination was noted in seven patients. It was, however, impossible to conclude if this was a consequence of the vaccination or of the CF disease itself.

4. Discussion

Results of a study on the immune response to pandemic 2009 A/H1N1 influenza vaccine in a large cohort of 122 patients with

CF are presented. After immunization, only 85% of the patients were seroprotected and 69% had seroconverted. Non-adjuvanted vaccine and low body mass index <−1.2 were independently associated with lower response to vaccination in non-transplanted patients.

This study addresses a clinically important question in the care of patients with CF. Until now, only scarce data have been available about immunogenicity and the clinical protection conferred by influenza vaccination in CF patients. Six studies [5–10] using different vaccines reported satisfactory serological antibody response in a total of 179 CF patients [8]. Two studies compared an intranasal live attenuated influenza A vaccine to an intramuscular inactivated trivalent influenza vaccine [6,8]. Two others compared a subunit influenza vaccine to a split virus influenza vaccine [5] or a trivalent virosomal influenza vaccine [9]. A single study compared patients with CF with healthy volunteers [7]. Our study demonstrates in a relatively large sample of CF patients representative of the CF population in terms of age distribution that not all the patients have an adequate response to influenzae vaccination and that malnutrition may be a risk factor for low immunization.

4.1. Clinical efficacy and pandemic 2009 A/H1N1 influenza vaccination

All the patients were followed at least every 3 months and were systematically questioned about influenza-like illness. They were instructed to call the CF center in case of any abnormal symptoms. It is therefore very improbable that we missed a symptomatic infection. Although we observed no evidence of symptomatic influenza infection nor worsening in the course of the CF respiratory disease in the vaccinated cohort during a follow-up of 1 year, our study was not able to assess clinical protection of pandemic vaccination. The absence of a control non-vaccinated group and the possibility of missed asymptomatic infections prevent any formal conclusion, especially in view of the very mild epidemic that followed [16,17]. Moreover, the absence of data on cell-mediated immunity limits the interpretation of the low immune response. Therefore, as the other influenza vaccine studies in the CF population [5–9] also did not evaluate clinical efficacy, the question of whether an annual influenza vaccination is clinically beneficial for people with CF remains unanswered [10].

4.2. Immune response to pandemic 2009 A/H1N1 influenza vaccination

Nearly one-third (31%) of the patients did not achieve seroconversion after vaccination and 15% were not seroprotected, including 10% of the non-transplanted patients. This was also the case for seasonal flu vaccination as 29% of the patients were not seroprotected against the seasonal flu at the onset of the epidemic period. One other study in a small cohort of 33 young CF patients reported a similar level of seroconversion after pandemic influenza A/H1N1 vaccination [18]. The importance of these findings lies in its contrast with studies in healthy populations which demonstrate excellent immunogenicity of the pandemic 2009 A/H1N1 vaccine for more than 95% of the young adults and 98% of the children and adolescents [19–21].

Similarly, persistent seroprotection at 10 months was only found in 50% of the patients who accepted a long term follow-up sample. Although only 75 of the 122 patients were tested, this result of poor long term seroprotection is in contrast to another study in a healthy population which showed persistence of HI antibodies in more than 95% of the patients [21].

Altogether these observations suggest a decreased immune response after pandemic influenza vaccination in our population with CF. We therefore questioned the risk factors of such a poor response.

4.3. Risk factors for a low immune response to pandemic 2009 A/H1N1 influenza vaccination in CF patients

Three factors were associated with a lower response to vaccination: lung transplantation, use of a non-adjuvanted vaccine and poor nutritional status.

The risk of serocoversion failure was 20 times higher in the transplanted versus the non-transplanted patients (OR: 20, 95% CI, 4–200). This was expected due to immune suppression and is in line with most published data, which show a diminished humoral response to vaccination in lung transplant recipients [22].

Both univariate and multivariate analysis adjusted on age demonstrated that non-adjuvanted vaccine remained significantly associated with the risk of poor immune response, thereby indicating that it is per se a risk factor for low immunity. This result is supported by several studies in the non-CF population also showing decreased immunogenicity of the non-adjuvanted vaccine [19,21,23].

Poor nutritional status as assessed by a BMI Z-score in the lowest quartile (<−1.2) increased the risk of seroconversion failure by a factor of 3.3 (1–10), and only half of the patients in this quartile seroconverted. Few studies have looked at the effect of malnutrition on vaccine response. This risk factor of low immune response has been mainly studied in elderly patients for vaccines against HBV, smallpox, and measles [24–26]. Protein energy malnutrition is known to decrease lymphocyte proliferation, reduce cytokine release, and lower the antibody response to vaccine [24]. It also prevents the differentiation of monocyte-derived dendritic cells and therefore decreases antigen-specific T-cell proliferation [26,27]. One recent study in the CF population also reported lower Z-scores for BMI in hepatitis B booster non-responders [28]. Studies on larger CF populations are necessary to fully evaluate the potential link between malnutrition and vaccine responsiveness in this specific population.

We did not show that early childhood was a risk factor for low immune response, a result however possibly due to insufficient power. Age was analyzed as a 2-class variable (<9 years as the reference class) as patients younger than 9 years were considered to be at risk of lower immune response to pandemic A/H1N1 vaccine according to the French official recommendations [13]. However, we do not think that this is an appropriate cut-off. We therefore adjusted for age to take into account the heterogeneity of the population of non-transplanted patients vaccinated with the non-adjuvanted vaccine, encompassing patients <24 months who required the non-adjuvanted vaccine and older patients who chose to receive this form of vaccine, We did not find any correlation between the seasonal flu antibody titer and the humoral response to the 2009 A/H1N1 flu vaccine, as already shown in healthy cohorts [29]. Previous vaccination against seasonal flu has been reported to hamper the development of cross-reactive immunity against influenza A viruses of novel subtypes [30]. It has been suggested that the patients may behave differently against the seasonal virus subtype and the pandemic H1N1 virus against which they were naive, by eliciting different heterosubtypic immunity [30]. All of our patients were vaccinated against the seasonal flu. This may be due to the enrolment in this vaccine study of patients particularly concerned by the immunization program, which is not representative of the CF population.

4.4. Is cystic fibrosis itself a risk factor for low immune response?

Nine of the 66 (14%) patients in which none of the observed risk factors of poor response were observed (i.e., vaccinated with the

adjuvanted vaccine and BMI Z-score >−1.2) failed to seroconvert. In these very particular cases, it is conceivable that CF itself impaired the response to specific antigenic stimulation by vaccination. CFTR expression has been demonstrated in lymphocytes and monocytes but so far this has not been linked to a functional role [31]. Several observations have offered evidence of a defective expression of CFTR in lymphocytes [31] and in invariant natural killer (iNK) cells in CF patients [32]. iNK cells provide B-cell help in both T-cell–dependent and T-cell–independent antibody responses [33]. Therefore one may speculate that defect in iNK cells might impair the response to specific antigenic stimulation, such as influenza vaccination.

Although only hypothetical for now, these very interesting observations point to a potential defect in the immune response of CF patients, at least for influenza vaccination and raise the question of a different influenza vaccination strategy. This includes vaccination of the entire household to limit virus spreading and antibody titer monitoring in at risk patients such as those patients with low BMI and transplant recipients. Immunization might be improved by either a more immunogenic vaccine or a different immunization schedule. Two doses of vaccines 1 month apart might be recommended to increase vaccine response. Adjuvanted vaccines might allow a better immune response but they may be associated with an increased rate of adverse events [34]. Finally, the attenuated live influenza vaccines could be a very interesting alternative in non-transplanted patients because they mimic natural infection by stimulating mucosal immune responses and specific T-cell repertoires and therefore could be more efficient than the traditional vaccines [34].

5. Conclusion

Our study strongly suggests that pandemic influenza A/H1N1 vaccination may induce a poorer immune response in CF patients than in the general population. Receipt of non-adjuvanted vaccine and low body mass index were independently associated with lower response to vaccination in non-transplanted patients. Under these circumstances, in view of the potential consequences of influenza on CF lung disease, we want to point out the importance of alternative strategies other than vaccination. Patients should be instructed that they may contract influenza even if vaccinated and be advised to take antiviral therapy such as oseltamivir at the least doubt during epidemic periods. These findings also underline the need to offer a more immunogenic vaccine to this high-risk population.

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Conflicts of interests

The authors declare no conflict of interest.

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Drs. Launay and Sermet-Gaudelus prepared the first draft of the manuscript. All authors contributed to the conduct of the study, the analysis and interpretation of the data, and the preparation of the manuscript. Dr. Sermet-Gaudelus had final responsibility for the decision to submit the manuscript for publication.

The statistical analysis of the data was conducted independently from the sponsor by co-author, Pierre-Yves Boelle from the Inserm U707. PY Boelle had access to all of the data used in the study and ran the analysis. He was not compensated for this work.

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Appendix A. Inserm Mucoflu study group members

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.06.010.

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