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Comparable humoral response after two doses of adjuvanted influenza A/H1N1pdm2009 vaccine or natural infection in allogeneic stem cell transplant recipients

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ABSTRACT

Background: The present study evaluated immunogenicity and tolerance of two-dose influenza A/H1N1pdm09 vaccination in allogeneic hematopoietic stem cell transplantation (HSCT) recipients, and compared the vaccine-induced humoral response to that triggered by natural infection in another group of HSCT patients.
Methods: Adult allogeneic HSCT recipients vaccinated with two doses of influenza A/H1N1pdm09 vaccine, separated by 3 weeks, and patients with proven influenza A/H1N1pdm09 infection were included. Antibody responses were measured by hemagglutination-inhibition assay 1) on days 0, 21, 42 and 6 months after the first vaccine injection in vaccinated patients and 2) before pandemic and after influenza A/H1N1pdm09 infection, in patients presented natural infection.
Results: At baseline, 3% of 59 recipients of adjuvanted vaccine and 0% of 20 infected patients were seroprotected (antibody titer $\geq$ 1/40). Seroprotection rate observed 42 days after vaccination was not different from that observed after natural infection (66% and 60% respectively, $p = 0.78$). In vaccinated patients, seroprotection rate increased significantly from 54% to 66% between day 21 and 42 ($p = 0.015$). Moreover, after 6 months, seroprotection rate in 21 vaccinated patients was similar to that observed in 10 infected patients evaluated at least 76 days after infection (D76–217) (60% and 81% respectively, $p = 0.2$). In multivariate analysis, no immunosuppressive treatment or chronic graft-versus-host disease (GVHD) and longer time between transplantation and vaccination/infection were associated with a stronger humoral response. The adjuvanted vaccine was safe with low rate of GVHD worsening.
Conclusion: In HSCT recipients, two doses of influenza A/H1N1pdm09 adjuvanted vaccine were safe and induced a humoral response comparable to that triggered by natural infection in these patients.

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1. Introduction

Influenza is a potentially serious infection in hematopoietic stem cell transplant (HSCT) recipients [1–5]. A mortality rate around 15% was reported in untreated patients, but recent data suggest an improvement of outcome for patients treated with neuraminidase inhibitors [3,5]. In a large prospective study of allogeneic HSCT recipients with influenza A/H1N1pdm2009 infection, 11% of patients with low respiratory tract disease required mechanical ventilation and 6% died from influenza infection or its complications [6].

Vaccination by an inactivated vaccine is the main prophylactic approach for influenza infection, but appears to be less effective in immunocompromised patients such as HSCT recipients, particularly during the first months after transplantation, or in patients with graft-versus-host disease (GVHD) receiving immunosuppressive treatments [7–12]. In this population, alternative modalities of vaccination have been evaluated such as the use of a second dose of vaccine, which allows an enhanced humoral response in some studies [7,13–15], and only marginal effect in others [16]. Moreover, in 2009, the emergence of a pandemic influenza virus has prompted the use of oil-in-water-emulsion adjuvant to enhance influenza vaccine immunogenicity [17]. Therefore, the French Health Authorities recommended the use of two doses of the adjuvanted pandemic influenza A/H1N1pdm09 vaccine in allogeneic HSCT recipients [18].

In this prospective study, we evaluated in allogeneic HSCT the immunogenicity and the safety of two doses of influenza A/H1N1pdm09 vaccine. The safety assessment was particularly focused on the potential risk of GVHD worsening with the adjuvanted vaccine which potentially could trigger an immune reaction against the recipient. To further evaluate the effect of these vaccination modalities, we compared the humoral response observed after adjuvanted vaccination to that observed after influenza A/H1N1pdm09 virus infection in HSCT patients included retrospectively. The primary objective was to compare humoral response observed after vaccination by 2 doses of influenza A/H1N1pdm09 adjuvanted vaccine and natural infection in allogeneic HSCT recipients.

2. Patients and methods

2.1. Study design

This observational study was conducted in 8 centers of the "Société Française de Greffe de Moëlle et Thérapie Cellulaire.

Vaccinated patients were prospectively included if they were older than18 years of age, and between 3 months and 5 years after allogeneic hematopoietic stem cell transplantation. Main exclusion criteria were: relapse of hematological disease, immunoglobulin infusion in the last 3 months, seasonal influenza vaccination within 3 weeks before first vaccine dose, on-going fever or infectious disease, allergy to egg or other components of the vaccine. Patients received two intramuscular injection (in deltoid muscle) of monovalent influenza A/H1N1pdm09 vaccine administered 21 days apart. The day of inclusion in the study was the day of the first vaccine administration.

Influenza-infected patients older than 18 years followed in our centers were eligible for the study if a pre-epidemic frozen serum sample was available. Post-infection sera were sampled at least 21 days after infection, whenever the patients came for a follow-up post-transplant visit. The day of sampling with positive PCR for diagnosis was considered as day 0.

Patients gave their oral consent to participate in the study as required for observational study. The protocol followed the Declaration of Helsinki and French law.

2.2. Vaccines

Fifty nine patients received an inactivated split-virion preparation of the influenza A/California/07/2009 (H1N1) strain containing 3.75 μg of hemagglutinin and AS03 adjuvant (Pandemrix®, GSK, UK). However, 11 patients received a non-adjuvanted vaccine (Panenza® [15 μg of hemagglutinin], Sanofi-Pasteur, Lyon, France) according to the choice of the physician, particularly in patients with active GVHD.

2.3. Safety assessment

Vaccinated patients recorded occurrence and severity of local or general reactions and any unsolicited adverse events during 21 days after each injection. Clinical or biological data about potential onset or worsening of GVHD were also collected.

2.4. Laboratory assays

2.4.1. Quantitative detection of influenza A/H1N1pdm09 antibodies

Humoral response to influenza A/H1N1pdm09 was evaluated on frozen sera using an hemagglutination-inhibition (HI) assay modified from Kendal et al. [19]. Briefly, after treatment with receptor destroying enzyme, two-fold dilutions of serum beginning 1/10, were tested against 4 hemagglutinin units of antigen (Panenza®, Sanofi-Pasteur, Lyon, France) on human O rhesus negative red blood cells. The titer of hemagglutination-inhibiting (HI) antibodies was defined as the reciprocal of the highest serum dilution that completely inhibited hemagglutination.

In all vaccinated patients, sera were obtained and tested prior to vaccination (D0) and 21 days after each vaccine injection (D21, and D42); in 21 of these patients, sera could be obtained and tested at 6 months post-immunization (M6). In infected patients, available frozen sera sampled before the onset of symptoms and at least 21 days after the diagnosis of infection were tested.

Immunogenicity was evaluated at each time point using the standard HI requirements used by European regulatory authorities for evaluation of influenza vaccines (EMEA) [20–22]. Seroprotection was defined as antibody titer $\geq$ 1/40 and seroconversion as a pre-vaccination/infection titer < 1/10 and a post-vaccination/infection antibody titer $\geq$ 1/40 or a pre-vaccination/infection titer $\geq$ 1/10 and at least a four-fold increase after vaccination/infection. Geometric mean of antibody titers (GMT) was also calculated. The 3 endpoints (with 95% confidence intervals) were (1) seroprotection rate (percentage of patients with antibody titer $\geq$ 1/40 before and after vaccination/infection) (2) seroconversion rate (percentage of patients who seroconverted after vaccination/infection) and (3) GMT ratio (GMT post-vaccination or infection/GMT at D0).

2.4.2. Molecular detection of influenza A/H1N1pdm09 virus

H1N1pdm09 infection was diagnosed from nasopharyngeal secretions by real-time reverse transcription-PCR assay according to the National Influenza Center Northern-France protocol (Institut Pasteur, Paris, France) [23] or to the Centers for Disease Control protocol (CDC) [24]

2.4.3. Definitions

Myeloablative conditioning regimens included either high dose busulfan (dose > 8 mg/kg orally or intravenous equivalent) or high dose total body irradiation ($\geq$8 Gy fractioned dose), both associated

with cyclophosphamide. Regimens not meeting these criteria were classified as reduced intensity conditioning (RIC).

Acute and chronic GVHD were diagnosed on clinical and/or histological grounds, based on standart criteria [25,26]. In patients who received prophylactic treatment of GVHD at the time of study entry, this treatment consisted of cyclosporine in most cases. In patients who received curative treatment of GVHD at the time of study entry, this treatment included corticosteroids in most cases. Worsening of GVHD was defined as a deterioration of symptoms in a target organ already involved or appearance of symptoms in a new target organ.

2.4.4. Statistical analysis

For seroprotection and seroconversion rates, exact confidence intervals (CI) were calculated. For GMT and GMT ratio95% confidence intervals were computed by taking the exponent of the mean and of the lower and upper limits of the 95% CI of the log-transformed titers.

The seroprotection and seroconversion rates at day 21 and day 42 were compared using the Mac Nemar's test. GMT at day 21 and day 42 were compared using Student's *t*-test for paired data.

Patient characteristics and immunogenicity data expressed as continuous variables were compared between the different groups using Kruskal–Wallis global tests; subsequent pairewise multiple comparisons used the Bonferroni-protected (at 0.017 level) Conover–Iman procedure as implemented in Xlstat 2.08 (Addinsoft TM). Comparisons of characteristics of patients described by qualitative variables used Fisher's exact tests.

A multivariate stepwise logistic model (level of significance 0.15 for entry, 0.05 for staying) was used to adjust for covariates. Analysis of factors associated with GMT ratio was performed using univariate and multivariate stepwise linear regression model of logarithm of titer ratio, using the same thresholds. The analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Patient characteristics

Ninety HSCT recipients were included in the study: 59 were vaccinated with an adjuvanted vaccine and received the first dose of vaccine between November 2009 and February 2010; 20 presented proven influenza A/H1N1pdm09 infection between October 2009 and March 2010. Eleven patients who received the non-adjuvanted vaccine were also included in the study but only for the safety analysis.

The demographic profiles and the clinical characteristics of the study patients are described in Table 1. Patients vaccinated with adjuvanted vaccine were older; the interval between transplantation and inclusion in the study was shorter in patients vaccinated with adjuvanted vaccine than in infected patients; the total lymphocyte count at inclusion was higher in patients vaccinated with the adjuvanted vaccine than in infected patients. Noteworthy, none of the vaccinated patient became influenza-infected.

In the group of 20 infected patients, the most frequent symptoms of influenza were high fever (85% of the patients), cough (85%), nasal discharge (60%) and myalgia (40%). Sixty percent of the patients presented a low respiratory tract infection but there was no acute respiratory distress syndrome. Coinfections were frequent, in particular with other respiratory viruses (coronavirus, $n=2$; rhinovirus, $n=3$; RSV, $n=1$; parainfluenza virus, $n=2$; adenovirus, $n=1$) and CMV ($n=1$). Nineteen patients received oseltamivir. Nineteen patients recovered from their influenza infection; one patient died from leukemia relapse while still excreting influenza A/H1N1pdm09 virus despite antiviral treatment with oseltamivir and zanamivir. Among 18/20 infected patients with available data about prior influenza A/H1N1pdm2009 vaccination, only one received one dose of the pandemic vaccine.

3.2. Safety of the vaccine

Four patients did not receive the second vaccine injection due to the occurrence of adverse events after the first dose: 3 with the adjuvanted vaccine and one with the non-adjuvanted vaccine ($p=1$). Local and general adverse events reported after vaccination were significantly more frequent in patients receiving the adjuvanted vaccine as compared to the non-adjuvanted one: 77% vs. 27% ($p=0.004$) for local side adverse events and 58% vs. 18% ($p=0.04$) for general adverse events. Worsening of chronic GVHD occurred in 4 patients: 3 after the adjuvanted vaccine and 1 after the non-adjuvanted vaccine ($p=1$).

3.3. Humoral response after 1 and 2 doses of influenza A/H1N1pdm09 adjuvanted vaccine

The baseline seroprotection rate and GMT of the 59 patients vaccinated with the adjuvanted vaccine were 3% and 7.2. The seroprotection rate increased from 53% at D21 after the first injection to 66%, after the second one at D42 ($p=0.015$); likewise the seroconversion rate increased from 51% at D21 to 66% at D42 ($p=0.007$) and the GMT from 34 at D21 to 96.6 at D42 ($p<0.0001$). In a subgroup of 21 patients, specific antibody titers were also evaluated 6 months after vaccination. Fig. 1 shows the baseline and rise of antibody titers in serum of these 21 patients at the four time points. Thus, antibody titers 6 months after vaccination were lower compared to D42, but were however maintained at higher values compared to D21 or baseline.

3.4. Humoral response comparison between adjuvanted vaccination and infection

The humoral response to influenza A/H1N1pdm09 measured after 2 doses of adjuvanted vaccine in 59 patients was compared to that observed after natural infection in 20 patients (Table 2). At baseline, no differences were observed in seroprotection rates or GMT between the 2 groups. The seroprotection rates were 66% in vaccinated patients at D42 and 60% in infected patients ($p=0.78$). Similar results were observed for seroconversion rates (66% vs. 60%; $p=0.78$), GMT (96.6 vs. 49.5; $p=0.09$) and for GMT ratios (13.6 vs. 9.1; $p=0.27$) in vaccinated and infected patients, respectively.

Since the range of time intervals between molecular diagnosis of influenza infection and humoral response evaluation was large (29–217 days, median value, 75), a more accurate analysis was performed by comparing 2 by 2 the humoral responses between 4 sub-groups: (a) the humoral response at D42 after vaccination ($n=59$) compared to the response obtained from D29 to D75 after infection ($n=10$) and (b) the late humoral response at M6 after vaccination ($n=21$) compared to the response obtained from D76 to D217 after infection. ($n=10$). No significant differences were observed for the three parameters in early or late humoral responses between the two groups (Table 3).

3.5. Factors associated with influenza humoral response

All the 79 patients, vaccinated with the adjuvanted vaccine or infected, were included in a univariate and multivariate analysis in order to identify the factors associated with the anti-influenza humoral responses in these settings. Results of the univariate analysis of the predictive factors for seroprotection and GMT ratio

Table 1
Patient demographics and clinical characteristics.

Characteristics	All patients $N=90$	Influenza infection $N=20$	Adjuvanted vaccine $N=59$	Non-adjuvanted vaccine $N=11$	p-Value[a]
Sex, n (%) of men	49 (54)	13 (65)	30 (51)	6 (60)	0.53
Median age (min–max), years	51 (20–69)	38 (22–56)	55 (20–69)	43 (21–59)	<0.0001[*]
Underlying hematological disease, n (%)					
Acute leukemia	35 (39)	7 (35)	23 (39)	5 (45)	
Lymphoma or CLL	26 (29)	7 (35)	16 (27)	3 (27)	
Multiple myeloma	7 (8)	2 (10)	4 (7)	1 (9)	0.73
Myelodysplastic syndrome	17 (19)	2 (10)	14 (24)	1 (9)	
Others	5 (5)	2 (10)	2 (3)	1 (9)	
Source of hematopoietic stem cells, n (%)					
Bone marrow	26 (29)	4 (20)	17 (29)	5 (45)	
Peripheral blood stem cells	59 (65)	13 (65)	41 (69)	5 (45)	0.082
Cord blood	5 (6)	3 (15)	1 (2)	1 (10)	
Myeloablative conditioning regimen, n (%)	36 (40)	8 (40)	22 (37)	6 (54)	0.46
HLA-related donor, n (%)	44 (49)	11 (55)	30 (51)	3 (27)	0.32
Previous or ongoing acute GVHD (grade ≥ II), n (%)	28 (32)	6 (30)	18 (31)	4 (36)	0.37
Chronic GVHD at inclusion, n (%)	34 (38)	10 (50)	18 (31)	6 (55)	0.14
Median time between transplantation and study inclusion (interquartile range), days	417 (204–978)	755 (378–1271)	330 (153–745)	637 (211–978)	0.049[**]
Immunosuppressive treatment at inclusion, n (%)	54 (60)	12 (60)	33 (56)	9 (82)	0.26
Total lymphocytes, median (interquartile range), 10^9/mL	1140 (800–1700)	900 (500–1300)	1300 (860–2400)	1070 (600–2950)	0.035[***]

GVHD: graft-versus-host disease; CLL: chronic lymphocytic leukemia.
[a] p-Value of the global comparison between the three groups of patients.
[*] The difference was significant between adjuvanted vaccine and both non-adjuvanted vaccine ($p<0.007$) and influenza infected patients ($p<0.0001$)
[**] The difference was significant between adjuvanted vaccine and influenza infected patients ($p<0.017$)
[***] The difference was significant between adjuvanted vaccine and influenza infected patients ($p<0.01$).

are shown in Table 4. Seroprotection rate was positively associated with a longer delay between transplantation and inclusion, lymphocyte count >1000/mm^3 and myeloablative conditioning regimen; and was negatively associated with a chronic GVHD and treatment by immunosuppressive drugs at inclusion. Chronic GVHD and treatment by immunosuppressive drugs at inclusion was associated with lower GMT ratio. Interestingly, the type of immunization (infection or vaccination), included in the analysis as a variable, was not associated to influenza humoral response.

In the multivariate analysis, treatment by immunosuppressive drugs at inclusion was associated with a lower seroprotection rate (odd ratio: 0.17 (95 CI%: 0.04–0.66), $p=0.007$). Moreover, a chronic GVHD and a short delay between transplantation and inclusion (<260 days) were associated with a lower GMT ratio (Table 5).

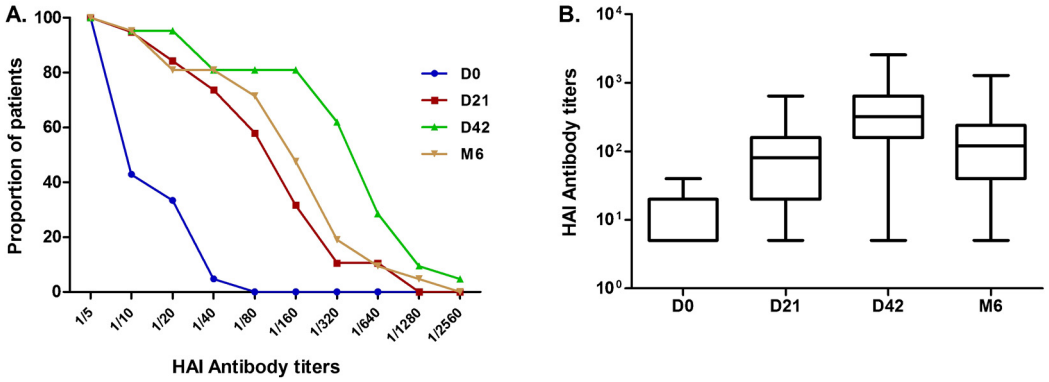

Fig. 1. Humoral response induced by the adjuvanted influenza A/H1N1pdm2009 vaccine in 21 HSCT recipients. Hemagglutination-inhibition (HAI) antibody titers in 21 patients vaccinated with two doses of adjuvanted influenza A/H1N1pdm2009 vaccine, before vaccination, at day 21, day 42 and at 6 months. A. Reverse cumulative distribution curves of antibody titers; B. Antibody titers (log10 transformed) represented in plots, the median titer (horizontal bar), minimal and maximal titers (error bars).

Table 2
Comparison of the humoral responses against influenza A/California/07/2009 (H1N1) after administration of two doses of adjuvanted vaccine or natural infection (HI).

	Adjuvanted vaccine	Natural infection	*p*-Value[a]
Pre-vaccination or pre-infection			
Number of tested patients	59	20	
Geometric mean titer (GMT) (95% CI)	7.2 (6.1–8.8)	5.5 (5.0–6.3)	0.103†
Seroprotection rate (%) (95% CI)	3 (0–12)	0 (0–16.8)	0.99
Post-vaccination (day 42) or post-infection			
Number of tested patients	56	20	
GMT (95% CI)	96.6 (55.1–156.4)	49.5 (26.5–104.1)	0.09†
Seroprotection rate (%) (95% CI)	66 (52–78)	60 (36–81)	0.78
Seroconversion rate (%) (95% CI)	66 (52–78)	60 (36–81)	0.78
GMT ratio (95% CI)	13.6 (8.3–21.2)	9.1 (4.9–19.3)	0.27†

[a] Fisher's exact test except for † (Kruskal-Wallis).

Table 3A
A Comparison of early humoral response: D42 after administration of 2 adjuvanted vaccine doses or D29-D75 after natural infection.

	Adjuvanted vaccine	Natural infection	*p*-Value[a]
Pre-vaccination or pre-infection			
Number of tested patients	59	10	
Geometric mean titer (GMT) (95% CI)	7.2 (6.1–8.8)	5.0 (5.0–5.0)	†0.06
Seroprotection rate (%) (95% CI)	3 (0–12)	0 (0–0)	0.5
Post-vaccination (day 42) or post-infection			
Number of tested patients	56	10	
GMT (95% CI)	96.6 (55.1–156.4)	60.6 (18.2–200.8)	†0.3
Seroprotection rate (%) (95% CI)	66 (52–78)	60 (26–88)	0.7
Seroconversion rate (%) (95% CI)	66 (52–78)	60 (26–88)	0.7
GMT ratio (95% CI)	13.6 (8.3–21.2)	12.1 (3.65–40.1)	†0.7

Seroprotection rate is defined as the percentage of patients with HI titer ≥ 1/40; seroconversion rate as the percentage of patients with a pre-vaccination/infection HI titer < 1/10 and a post-vaccination/infection titer ≥ 1/40, or showing a significant increase in antibody titer defined as a pre-vaccination/infection titer ≥ 1/10 and at least a fourfold increase in post-vaccination/infection titer; geometric mean titer ratio or seroconversion factor as the geometric mean of the within-subject ratios of the post-vaccination/infection reciprocal HI titer to the day 0 reciprocal HI titer.

[a] Fisher's exact test except for † (Kruskal–Wallis).

Table 3B
Comparison of late humoral response: M6 after administration of 2 adjuvanted 543 vaccine doses or D76-D217 after natural infection.

	Adjuvanted vaccine	Natural infection	*p*-Value[a]
Pre-vaccination or pre-infection			
Number of tested patients	21	10	
Geometric mean titer (GMT) (95% CI)	8.9 (6.5–12.3)	6.0 (4.7–7.6)	†0.14
Seroprotection rate (%) (95% CI)	4 (0.1–24)	0 (0–0)	0.4
Post-vaccination (M6) or post-infection			
Number of tested patients	21	10	
GMT (95% CI)	101.7 (56.0–184.7)	40.4 (19.6–83.5)	†0.06
Seroprotection rate (%) (95% CI)	81 (58–95)	60 (26–88)	0.2
Seroconversion rate (%) (95% CI)	81 (58–95)	60 (26–88)	0.2
GMT ratio (95% CI)	11.38 (6.7–19.3)	6.7 (3.3–13.7)	†0.33

Seroprotection rate is defined as the percentage of patients with HI titer ≥ 1/40; seroconversion rate as the percentage of patients with a pre-vaccination/infection HI titer < 1/10 and a post-vaccination/infection titer ≥ 1/40, or showing a significant increase in antibody titer defined as a pre-vaccination/infection titer ≥ 1/10 and at least a fourfold increase in post-vaccination/infection titer; geometric mean titer ratio or seroconversion factor as the geometric mean of the within-subject ratios of the post-vaccination/infection reciprocal HI titer to the day 0 reciprocal HI titer.

[a] Fisher's exact test except for † (Kruskal–Wallis).

4. Discussion

This is the first study in allogeneic HSCT recipients, comparing immunogenicity induced by influenza A/H1N1pdm09 vaccination and natural infection. It shows that vaccination by two doses of adjuvanted influenza A/H1N1pdm09 vaccine can elicit a specific humoral response, comparable to that achieved after infection. Indeed, two of the three EMEA serological criteria [20,21,27] for assessment of vaccine immunogenicity in immunocompetent individuals (seroconversion rate > 40% and GMT ratio > 2.5) were fulfilled at D42, and the third one was nearly reached (seroprotection rate at 66% instead of 70% as required). Other studies in allogeneic HSCT recipients showed similar responses using the same vaccination regimen [13,14,28] and higher responses were also reported [15,29]. Nevertheless, these studies included patients with different clinical characteristics, in particular longer period of time between transplantation and vaccination.

The present study highly suggests the usefulness of a second dose of vaccine in HSCT patients. At the beginning of the 2009 influenza pandemia, it was expected that two doses of adjuvanted vaccine would be necessary to induce a substantial humoral response even in immunocompetent subjects, but a single injection was subsequently shown to be sufficient [30]. By contrast, in different types of immunocompromised patients, several studies reported that second injection was necessary to achieve antibody titers comparable to those obtained in control subjects after one injection [31,32]. In the present study, HSCT recipients were planned to receive the 2 doses of vaccine recommended by the French guidelines and the recent guidelines of the 4th ECIL [18,33]. The significant increase of specific antibody titer between the first

Table 4
Univariate analysis of predictive factors of humoral response after adjuvanted vaccination (D42) or natural infection by H1N1pdm09.

Variable	Seroprotection		GMT ratio	
	Odds-ratio (95% CI)	*p*-Value	Ratio (95% CI)[a]	*p*-Value
Groups				
Infected	1		1	
Vaccinated	1.3 (0.45–3.72)	0.63	1.49 (0.59–3.74)	0.38
Age ≥ 50 years	1.14(0.44–2.92)	0.78	1.33 (0.59–3.03)	0.47
Sex male	0.90 (0.35–2.32)	0.83	1.28 (0.57–2.91)	0.54
Delay since transplant (d)				
<260	1		1	
260–730	2.86 (0.91–9.02)	0.07	2.41 (0.92–6.30)	0.08
>730	5.09(1.45–17.9)	0.01	2.46 (0.92–6.55)	0.07
Grade II–IV aGVHD	0.87(0.31–2.43)	0.78	1.00(0.40–2.50)	0.92
Chronic GVHD at inclusion	0.26 (0.09–0.71)	0.01	0.30(0.13–0.67)	0.004
Graft source				
Periph. blood	1		1	
Cord blood	0.53 (0.07–4.08)	0.54	0.97(0.14–6.89)	0.95
Bone marrow	0.98 (0.33–2.9)	0.97	0.91 (0.15–5.75)	0.92
HLA related donor	0.77(0.3–1.97)	0.58	0.70(0.32–1.57)	0.38
Immunosuppressive treatment at inclusion	0.18 (0.06–0.54)	0.01	0.29(0.14–0.64)	0.003
Myeloablative conditioning regimen	3.1 (1.07–9)	0.04	1.68 (0.26–3.81)	0.21
Lymphocytes ≥ 1000/mm^3	3.2(1.11–9.39)	0.03	2.14(0.89–5.16)	0.09

GVHD: graft versus host disease.
[a] Ratio of GMT ratios with respect to variable values.

and the second administration of adjuvanted vaccine highly suggests the interest of the second dose of vaccine. However, the design of this study cannot totally exclude a delayed response to the first vaccination. Furthermore, we showed that the response was durable since 17 out 21 patients remained seroprotected after 6 months. Of note, long-term humoral immunity induced by influenza vaccine in HSCT recipients had not been previously evaluated.

There is little information about antibody dynamics induced by natural influenza infection in humans [34–36]. Only a few studies compared the humoral immune response induced by influenza vaccination or natural infection and they were conducted in immunocompetent subjects [35–37]. Herein we show that the seroprotection rate, as well as the GMT of antibodies at day 42 and even at M6 were not statistically different between infected and vaccinated patients, suggesting that a regimen of two doses of adjuvanted vaccine is as efficient as natural infection in inducing specific humoral response in HSCT patients. Moreover, there is a tendency, although not significant, toward higher GMT at long term in vaccinated patients as previously reported in non-HSCT recipients [36]. To our knowledge, our data are the first showing that the humoral response after vaccination is at least as good as the response induced by natural infection in HSCT recipients and argue for the use of 2 doses of adjuvanted vaccines in these patients.

Factors influencing the influenza humoral response in HSCT have been previously reported in studies conducted to evaluate vaccine response [7,10,29,38]. For the analysis of factors influencing the humoral response to influenza, we considered the 79 patients as a single group and vaccination or infection as a variable factor. In multivariate analysis, our data showed that the humoral response is independent of the type of influenza immunization (i.e. natural infection or adjuvanted vaccine) but depends on several other parameters: time interval between transplantation and vaccination/infection, treatment by immunosuppressive drugs, presence of chronic GVHD at inclusion. These results must be considered when establishing a new schedule of vaccination in such immunocompromised patients. As previously reported the total lymphocyte counts were associated with the seroprotection rate, in univariate analysis [14]. However this association did not remain in the multivariate analysis. This can be explained because lymphocyte count highly depends on administration of immunosuppressive treatment.

Table 5
Multivariate analysis of predictive factors of humoral response after vaccination (D42) or natural infection by H1N1v.

Variable		GMT ratio	
		Ratio (95% CI)[a]	*p*-Value
Delay since transplant (d)	<260	1	
	260–730	63 (1.05–6.6)	0.04
	>730	75(1.09–6.8)	0.03
Chronic GVHD at inclusion		0.28 (0.13–0.6)	0.002

GVHD: graft versus host disease.
[a] Ratio of GMT ratios with respect to variable values.

The use of a non-adjuvanted vaccine in a group of 11 patients allowed comparing the intensity and frequency of adverse events between the two types of vaccines. Despite the poor number of patients vaccinated with the non adjuvanted vaccine, we observed that, as expected, the adverse events (with moderate intensity) were more frequent with the adjuvanted compared to the non-adjuvanted vaccine, but they were lower than previously reported [13,29]. The proportions of patients who did not receive the second dose due to adverse events were comparable in the two groups. The same was observed for the rates of chronic GVHD worsening, which remained low in both groups.

The main limitation of our study is the small number of infected patients and their retrospective inclusion that led to large time intervals between blood sampling before and after infection. However we considered HSCT recipients with proven H1N1pdm09 infection as the most relevant control group to evaluate new modalities of vaccination, despite the heterogeneity and the small number of patients included in this group.

In conclusion, in this study, vaccination with two doses of influenza A/H1N1pdm09 adjuvanted vaccine is safe in allogeneic HSCT recipients and allows the generation of a humoral response comparable to that triggered by natural infection. The humoral response observed after vaccination in this immunosuppressed population reaches 2 out of 3 EMEA criteria for evaluation of influenza vaccines in healthy subjects. The lack of seroprotection in some patients after natural infection or vaccination is most likely explained by a poor post-transplant immune recovery.

Authorship and disclosures

ND and AK were the principal investigators and take primary responsibility for the paper; BA participated to the design of the study. ND, BL, JOB, PA, MTR, AT, PR, recruited the patients; AK, NLC, JLG and BA performed the laboratory work for this study; AM performed the statistical analysis; ND, AK, PR and NLC wrote the paper.

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