Vaccine 30 (2012) 7522–7528

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Effect of two injections of non-adjuvanted influenza A H1N1pdm2009 vaccine in renal transplant recipients: INSERM C09-32 TRANSFLUVAC trial

Nicole Le Corre[a,b,1,2], Fréderic Thibault[b,c,1,2], Claire Pouteil Noble[d,e,1], Vincent Meiffrédy[f,1], Sameh Daoud[g,1], Remi Cahen[d,1], Isabelle Charreau[f,1], David Bottigioli[h,1], Cécile Dollinger[g,1], Jean-Pierre Aboulker[f,1], Brigitte Autran[a,b,i,1], Emmanuel Morelon[e,g,1,3], Benoit Barrou[b,c,*,1,3]

[a] *INSERM UMRS-945, Laboratoire Immunité et Infection, Groupe Hospitalier Pitié-Salpêtrière Paris, France*
[b] *Université Pierre et Marie Curie, Paris VI, France*
[c] *Service d'Urologie, Groupe Hospitalier Pitié-Salpêtrière, APHP, Paris, France*
[d] *Service de Néphrologie, Centre Hospitalier Lyon-Sud, Lyon, France*
[e] *Université de Lyon, France*
[f] *INSERM SC10, Hôpital Paul Brousse, Villejuif, France*
[g] *Service de Néphrologie, Transplantation, Immunologie Clinique, Hôpital Edouard Herriot, Lyon, France*
[h] *Unité de Recherche Clinique en Immunologie, Lyon Sud, France*
[i] *Département d'Immunologie, Groupe Hospitalier Pitié-Salpêtrière, APHP, Paris, France*

ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 3 October 2012
Accepted 13 October 2012
Available online 25 October 2012

Keywords:
2009 H1N1 pandemic
Influenza vaccine
Non-adjuvanted vaccine
Immunosuppressed patients
Kidney transplantation

ABSTRACT

Background: Enhancing vaccine immunogenicity in kidney transplant recipients, particularly against influenza, is required since the immunosuppression used to prevent graft rejection limits vaccine immunogenicity. We therefore investigated the immunogenicity and safety of a double dose non-adjuvanted vaccination regimen against influenza H1N1pdm2009 in kidney transplant adult recipients.
Methods: A prospective single-arm study was conducted including 121 renal transplant recipients under triple immunosuppressive regimen. Patients received 2 injections (day 0, day 21) of an inactivated, non-adjuvanted H1N1pdm2009 vaccine. Immunogenicity (hemagglutination-inhibition [HI] antibodies and anti-hemagglutin [HA] specific T cells) was evaluated after one and two injections (day 21, day 42) and at 6 months (day 182).
Results: The seroprotection rate (HI antibody titer $\geq$ 1/40) was 19% at day 0 (n = 119), 53% at day 21 (n = 118), 60% at day 42 (n = 116) (p = 0.013; day 42 vs. day 21) and 56% at day 182 (n = 113). The seroconversion rate was 24% and 32%, the geometric mean fold rise was 3.7 and 4.6 after the first and second injections, respectively. T-cell immunity to the H1N1pdm2009 vaccine showed a two-fold increase from baseline, though not statistically significant, in H1N1pdm2009-HA-specific CD4+ and CD8+ T cells in 34% and 48% of cases, respectively. No rejection episodes related to vaccination were observed while the donor-specific antibodies and creatinine clearance remained unchanged throughout the study.
Conclusion: Administration of two doses of the non-adjuvanted influenza H1N1pdm2009 vaccine in renal transplant patients is safe and induces a significant seroprotection, not strong enough yet to meet European or US requirements for adults below 60 years, but comparable to seroprotection levels usually observed in the non immunosuppressed elderly population or conferred by a single dose of adjuvanted vaccine in solid organ transplant recipients. These results provide useful indications for future strategies required to improve immunogenicity of vaccines against influenza in transplanted patients.

1. Introduction

Influenza infection in transplant recipients is associated with high morbidity, mortality and graft rejection [1–3]. Therefore, guidelines recommend annual vaccination against seasonal influenza for patients with solid organ transplantation [4–6].

Vaccination in transplant recipients is a matter of controversy since a compromise has to be found between a strong immunosuppressive regimen to prevent graft rejection, which

* Corresponding author at: Service d'Urologie – Unité de Transplantation, Groupe Hospitalier Pitié-Salpêtrière, 47–83, Boulevard de l'Hôpital, 75651 Paris Cedex 13, France. Tel.: +33 01 42 17 71 14; fax: +33 01 42 17 71 93.
E-mail address: benoit.barrou@psl.aphp.fr (B. Barrou).
[1] For The INSERM C09-32 TRANSFLUVAC study group, see Appendix A.
[2] The first two authors contributed equally to the study.
[3] The last two authors contributed equally to the study.

0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2012.10.047

however limits vaccine immunogenicity, and a strongly immunogenic vaccine to elicit protective immunity without triggering graft rejection. Data on the efficacy of seasonal influenza vaccination in kidney transplant recipients are conflicting. Some studies report a suboptimal response to influenza vaccine in these patients [7–10], while other showed no differences with healthy persons [11–13]. When the H1N1pdm2009 virus appeared, most countries recommended using two injections of either an adjuvanted or a non-adjuvanted inactivated monovalent A H1N1pdm2009 vaccine in immunocompromised individuals [14,15]. Studies demonstrated a weak immunogenicity of a single dose of the non-adjuvanted anti-H1N1pdm2009 vaccine in relatively small groups of kidney transplant recipients [16,17]. A very small study failed at demonstrating improvement after a second dose [18], while the addition of an adjuvant to improve immunogenicity remained controversial [19–24].

Altogether those studies used the criteria normally used to test influenza vaccines in immunocompetent populations under 60 years old, but consensus criteria have not been defined for the immunosuppressed population [25–27]. This latter group might be better compared with the elderly population in whom seasonal and pandemic influenza vaccines are highly recommended but poorly immunogenic and lower levels of the three reference criteria recommended in Europe have been judged acceptable.

To better investigate the immunogenicity and safety of two injections of a non-adjuvanted H1N1pdm2009 vaccine in kidney transplanted populations, which had been thus recommended in France, we conducted a prospective single-arm clinical trial evaluating these parameters after one and two injections as well as the immune memory at 6 months, in 121 renal transplant patients under maintenance therapy with triple immunosuppressive regimen.

2. Materials and methods

2.1. Vaccine

The non-adjuvanted monovalent A/H1N1pdm2009 inactivated split-virion vaccine from Sanofi-Pasteur SA was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York) generated from the A/California/07/2009 (H1N1) strain as recommended by World Health Organization [28], and contained 15 μg HA in each 0.5 mL dose.

2.2. Study design

This phase 2, prospective, single-arm, clinical trial was performed in three transplantation centers in France. Renal transplant patients received two intramuscular injections (in deltoid muscle) of influenza A H1N1pdm2009 vaccine administered 21 days apart. Patients eligible to participate in this study were 18–65 years of age, benefited of renal transplantation at least 6 months ago, with a creatinine clearance >20 mL/min, had stable renal function defined as serum creatinine variation <20% for the last three months, were receiving a triple immunosuppression regimen including steroids, calcineurin inhibitors (cyclosporine or tacrolimus) and IMPDH inhibitors (mycophenolate mofetil or mycophenolic acid).

Main exclusion criteria were: pregnancy, acute rejection episode during 3 months before inclusion, known human immunodeficiency virus (HIV) infection, on-going treatment for chronic hepatitis B or active hepatitis C, allergy to egg or other vaccine components, severe adverse events after prior administration of any influenza vaccine, multiple sclerosis, history of Guillain-Barré syndrome, fever at inclusion, influenza (virologically documented) during the last 6 months, contact with people infected with H1N1 influenza during the week prior to inclusion.

Written informed consent was obtained from each patient. The protocol (ClinicalTrials. Gov: NCT01086904) was conducted in accordance with the Declaration of Helsinki and French law, and approved by the local Ethics Committee ("Comité de Protection des Personnes Ile-de-France III", Paris).

2.3. Laboratory assays

Blood samples, for assessment of hemagglutination-inhibition (HI) antibodies were planned prior to vaccination (D0), 21 days after each vaccine injection (*i.e.*, day 21 (D21) and day 42 (D42) after first injection) and at day 182 (D182).

Specific antibody assays were performed at Sanofi-Pasteur Global Clinical Immunology Laboratory (Swiftwater, PA, USA). The titer of antibodies against the vaccine strain was measured in duplicate for each sample by a validated HI method according to the WHO Collaborating Center for Influenza, Centers for Diseases Control, Atlanta, USA [29].

Specific T cell-mediated responses was evaluated in a substudy of 29 patients from a single center using multiparametric intracellular cytokine staining (ICS) flow cytometry assay as previously described [30]. Thawed peripheral blood mononuclear cells (PBMCs) (above 85% viability) were stimulated for 5 h with pools of overlapping 12–18mer peptides covering the whole influenza A/California/07/2009 (H1N1) hemagglutinin (HA) (Eurogentec®, Liege, Belgium) together with brefeldin A and monensin (Sigma®); positive and negative control were staphylococcal enterotoxin B (SEB) and culture medium alone respectively. Cells were stained using anti-CD4-ECD (Beckman Coulter®), anti-CD8-APC-Cy7, anti-CD3-Pacific Blue, anti-CD40L-PE, anti-IFNγ-Alexa700, anti-IL2-APC, anti-TNFα-PCy7 (BD Bioscience®) and anti-MIP1β-FITC (RD Systems®) monoclonal antibodies. At least 1 million cells were analyzed on a Gallios® Flow Cytometer and with Kaluza® (Beckman Coulter®). Results were expressed as the sum of frequency of H1N1pdm2009-HA-specific CD4+ or CD8+ T cells producing IFNγ, IL2, TNFα, and MIP1β and/or expressing CD40L, after subtracting background values of negative controls.

2.4. Safety assessment

Patients were provided with diary cards to record the occurrence and severity (graded as mild, moderate or severe) of solicited local or general reactions and any unsolicited adverse events during 21 days after vaccination. Temperature was recorded daily during 7 days after vaccination.

Subjects having an influenza-like illness defined as temperature above 37.8 °C with at least one influenza-like symptom were evaluated with virological test.

All patients were tested for the presence of donor-specific anti-HLA antibodies on D0 and after the second injection on D42, using a highly sensitive solid phase detection assay (LABScreen Single Antigen Class I – Group 4 and Single Antigen Class II – Group 1; One Lambda Inc., Canoga Park, CA).

2.5. Statistical analysis

A sample size of 120 patients was needed according to the standard criteria for evaluation of influenza vaccines [31].

Immunogenicity was analyzed by the standard HI endpoints (with 95% confidence intervals) used by EMEA for evaluation of influenza vaccines [25–27]: (1) seroprotection rate (HI antibody titer $\geq$ 1/40), (2) seroconversion rate (pre-vaccination titer < 1/10 and a post-vaccination titer $\geq$ 1/40 or at least a four-fold increase in post-vaccination titer) and (3) geometric mean fold rise (ratio

of geometric mean titers (GMT) of post-vaccination and baseline antibody titers). For proportions of subjects with HI antibody, exact confidence intervals were built. For GMT and geometric mean fold rise, 95% confidence intervals were computed by taking the exponent ($\log_{10}$) of the mean and of the lower and upper limits of the 95% CI of the $\log_{10}$-transformed titers, and were compared using paired *t*-test. The seroprotection rates were compared using the Mac Nemar's test.

3. Results

3.1. Study patients

From November to December 2009, 121 renal transplant patients were enrolled and received a first injection of vaccine (Fig. 1); three patients did not receive the second injection (confirmed H1N1 influenza, $n = 1$; patient request after influenza-like episode without virological analysis, $n = 1$; patient request after fainting following first injection; $n = 1$). Patients' characteristics are described in Table 1.

Table 1
Demographic and clinical characteristics of the patients of the TRANSFLUVAC study.

Characteristics	$n = 121$
Sex, *n* (%) of men	89/121 (74)
Median age (min–max), years	50.9 (20–64)
Geographic origin, *n* (%)	–
Caucasian	103/121 (85)
African	6/121 (5)
Asian	2/121 (2)
Other	10/121 (8)
Median time since renal transplantation, (min–max), years	3.4 (0.5–21)
Anti-rejection regimen, *n* (%)	
Prednisone/prednisolone + tacrolimus + MMF	77/121 (64)
Prednisone/prednisolone + cyclosporine + MMF	44/121 (36)
Estimated creatinine clearance[a], median (min–max), mL/min/1.73 m^2	54 (23–127)
Coinfections, *n* (%)	
HBV	2/121 (2)
HCV	2/120 (2)
HIV	0
Received 2009 seasonal influenza vaccine, *n* (%)	61/121 (50)

MMF: mycophenolate mofetil/mycophenolic acid.
[a] Estimated with the modification of the diet in renal disease formula.

3.2. Immunogenicity

At baseline, 19% of patients had seroprotection (HI antibodies titers $\geq$ 1/40) against A/California/07/2009 (H1N1) with 23% seroprotected among the 61 patients who had previously received the seasonal influenza vaccine, compared to 16% among the 58 who did not received it ($p = 0.30$). Overall, the proportion of patients with seroprotection significantly increased to 53% at D21, 60% at D42 ($p = <0.0001$ for both) and remained at 56% on D182.

Seroconversion rates were 24% at D21, 32% at D42 and 30% at D182. GMT increased from 13 at baseline to 48 at D21, 59 at D42 and remained stable at 52 on D182. GMT significantly rise from baseline, was 3.7 at D21 and 4.6 at D42 ($p < 0.001$ for both) and was maintained at 4.0 on D182 (Table 2).

When comparing the humoral response after one and two doses of the vaccine, the seroprotection rate and GMT significantly increased between D21 and D42 ($p = 0.013$ and $p = 0.004$ respectively). Of note, eleven patients (10%) non seroprotected at D21 achieved HI titers $\geq$ 1/40 after the second dose of the vaccine. The evolution with time of the distribution of individual plots of antibody titers is presented in Fig. 2.

No difference in seroprotection rate was observed at D21 and D42 among the 61 patients who received previously the seasonal influenza vaccination compared to those patients who did not receive it (data not shown). Futhermore, no differences were

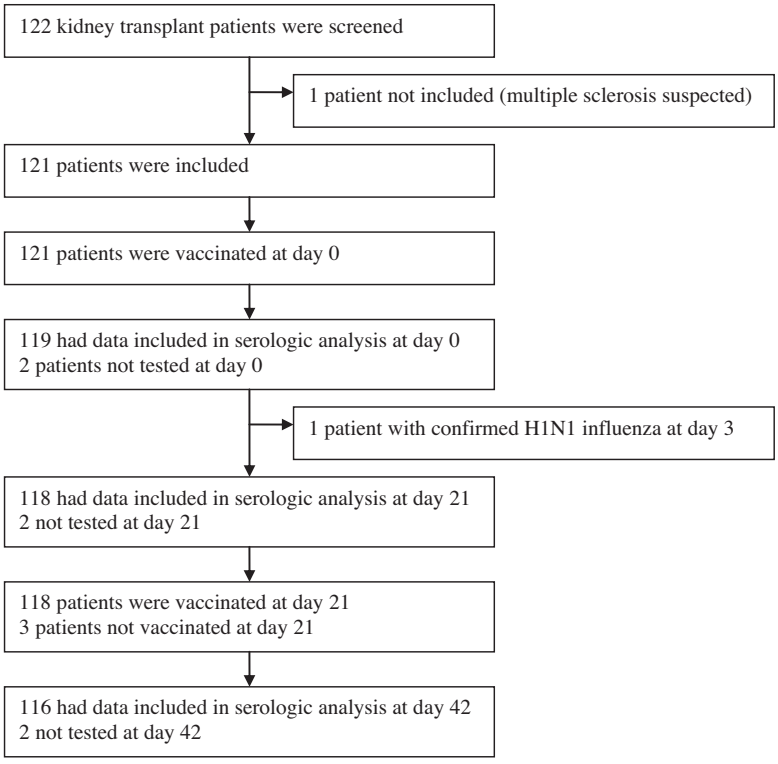

Fig. 1. Disposition of kidney transplant patients in the TRANSFLUVAC trial.

Table 2
Immune response in kidney transplant patients: hemagglutination inhibitory antibodies against A/California/07/2009 (H1N1).

	Day 0	Day 21	Day 42	Day 182
Number of tested patients	119	118	116	113
Geometric mean titer [95% CI]	12.7 (10.0–16.0)	47.6 (34.3–65.9)	59.0 (43.5–79.9)	52.2 (38.3–71.2)
Seroprotection rate, *n* (%) [95% CI]	23/119 (19) (13–28)	62/118 (53) (43–62)	70/116 (60) (51–69)	63/113 (56) (46–65)
Seroconversion rate, *n* (%) [95% CI]	NA	28/118 (24) (16–32)	37/114 (32) (24–42)	34/112 (30) (22–40)
Geometric mean fold rise [95% CI]	NA	3.7 (2.8–4.9)	4.6 (3.5–6.0)	4.0 (3.1–5.2)

NA: not applicable.
Seroprotection rate is defined as the percentage of patients with a HI antibody titer ≥ 1/40; seroconversion rate as the percentage of patients with a pre-vaccination HI titer <1/10 and a post-vaccination titer ≥ 1/40, or showing a significant increase in antibody titer defined as a pre-vaccination titer ≥ 1:10 and at least a fourfold increase in post-vaccination titer; geometric mean fold rise or seroconversion factor as the geometric mean of the within-subject ratios of the post-vaccination reciprocal HI titer to the Day 0 reciprocal HI titer.

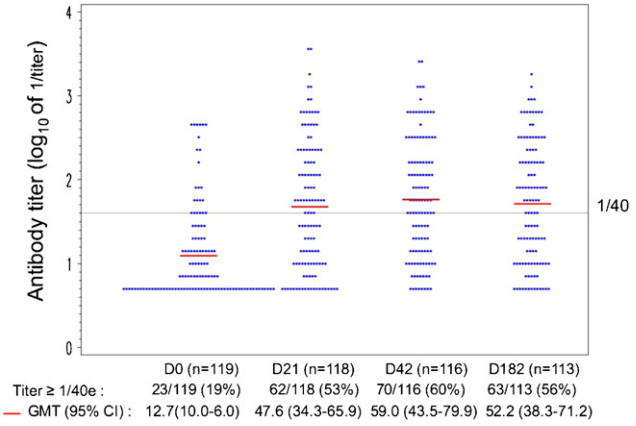

Fig. 2. Serum titers of hemagglutination inhibitory antibodies against A/California/07/2009 (H1N1). Antibody titers are expressed as $\log_{10}$ of 1/titer. At each time point, seroprotection rate, (percentage of patients with a HI antibody titer ≥ 1/40) and GMT (geometric mean titer) are indicated.

detected in seroprotection rate or GMT at D21 or D42 between patients receiving tacrolimus or cyclosporine (data not shown). Similarly, gender, time from renal transplantation and history of diabetic nephropathy were not associated to seroprotection. Contrarily, participant were significantly older in the group without seroprotection than with seroprotection at D21 and D42: mean age (standard deviation) were respectively 50 years-old (8) vs. 46 years-old (11) at D21 ($p = 0.04$) and 52 years-old (8) vs. 47 years-old (11) at D42 ($p = 0.007$).

The T cell immunity substudy was performed for 29 patients from a single center who were comparable to the rest of the study group except for the median time since renal transplantation (1.5 [0.8; 3.6] vs. 4.0 years [2.0; 7.2], respectively, $p = 0.0001$) and previous seasonal influenza vaccine (34% vs. 55%, $p = 0.05$). Overall, low T cell reactivity was observed throughout the study without significant increases in the frequencies of H1N1pdm2009-HA-specific CD4+ or CD8+ T cells producing IFNγ, IL2, TNFα, MIP1β or expressing CD40L, as shown in Table 3. Nevertheless, a 2-fold increase in frequencies of H1N1pdm2009-HA-specific CD4+ T cells and CD8+ T cells from D0 to any post-vaccination time point was observed in 10/29 (34%) and 14/29 (48%) vaccines, respectively. No correlation was observed between humoral and cellular responses yet.

3.3. Safety

The total follow-up was 56.3 patients-years. At least one injection-site reaction or general adverse event was reported for 42/121 patients (35%): injection-site reaction for 24/121 patients (20%) or general reaction for 28/121 patients (23%). Five immediate (in 4 patients) and 27 delayed (in 19 patients) solicited injection-site reactions were reported. The most frequent injection-site reaction was pain. No immediate and 52 delayed (28 patients) solicited general reactions were reported. The most frequent solicited general reactions were headache and asthenia. The majority of solicited adverse events were mild to moderate in intensity; most of them occurred after the first injection (Table 4).

One unsolicited injection-site adverse event was reported and 14 unsolicited general adverse events were reported for 9 patients, all graded as mild to moderate except one adverse event (fainting, $n = 2$; severe). Fifteen serious adverse events were reported; none related to the vaccination and all related to complications of diabetic foot, urinary infection, pulmonary embolism, acute bowel sub-occlusion, severe hypoglycemia, removal of surgical material, inguinal hernia treatment, steroid-induced diabetes, patellar tendon rupture, atrial fibrillation and glomerular renal graft rejection, evidenced 2.5 years post transplantation. The evolution was favorable after treatment.

The calculated creatinine clearance (MDRD formula) did not vary during the study (median from 54 to 55 mL/min/1.73 m^2 between D0 and D42).

On D0, 57% of the patients and 51% on D42 were tested positive for anti-HLA antibodies. At baseline, 5 (4.1%) had anti-class I donor-specific antibodies (DSA) with mean fluorescence index

Table 3
Cell-mediated response induced by non-adjuvanted influenza A H1N1pdm2009 vaccine in kidney transplant patients: median frequencies of H1N1pdm2009-HA-specific CD4+ and CD8+ T cell by intracellular cytokine staining flow cytometry assay.

	Day 0	Day 21	Day 42	Day 182	*n* (%) of 2-fold increase from D0 to D21, D42 or D182
Number of tested patients	29	28	28	29	29
CD4+ T cells, median [IQR],	0.157 [0.086; 0.228]	0.095 [0.056; 0.192]	0.106 [0.059; 0.202]	0.137 [0.081; 0.271]	10 (34%)
n (%) of 2-fold increase	NA	6 (21.4%)	5 (17.9%)	7 (24.2%)	
CD8+ T cells, median [IQR],	0.076 [0.040; 0.171]	0.060 [0.017; 0.116]	0.055 [0.016; 0.240]	0.107 [0.043; 0.154]	14 (48%)
n (%) of 2-fold increase	NA	7 (25%)	8 (28.6%)	12 (41.4%)	

NA: not applicable.
Results are expressed as the sum of frequency of H1N1pdm2009-HA-specifics CD4+ or CD8+ T cells producing IFNγ, IL2, TNFα, MIP1β and/or expressing CD40L, after subtracting the background value of cytokine production by unstimulated PBMCs.

Table 4
Solicited reports of injection-site and general adverse events after the injection of the influenza A H1N1pdm2009 vaccine.

	First injection, $n = 121$ n (%)	Second injection, $n = 117$ n (%)
Solicited injection-site reactions[a]		
Immediate (≤30 min)	2 (2)	3 (3)
Pain	2 (2)	1 (1)
Erythema	0	1 (1)
Edema	0	1 (1)
Delayed (>30 min)	20 (17)	7 (6)
Pain	11 (9)	6 (5)
Bruise	2 (2)	1 (1)
Erythema	3 (3)	0
Induration	2 (2)	0
Edema	2 (2)	0
Solicited general reactions[b]		
Immediate (≤30 min)	0	0
Delayed (>30 min)	33 (27)	19 (16)
Headache	14 (12)	7 (6)
Asthenia	9 (7)	4 (3)
Chills	3 (2)	3 (3)
Myalgia	3 (2)	2 (2)
Pyrexia	2 (2)	1 (1)
Arthralgia	1 (1)	1 (1)
Hyperhydrosis	1 (1)	1 (1)

[a] Intensity: mild, $n = 18$; moderate, $n = 3$; unknown, $n = 11$.
[b] Intensity: mild, $n = 45$; moderate, $n = 7$.

(MFI) ranging from 430 to 2425; 13 (10.7%) had DSA against class II antigens with MFI ranging from 303 to 8500. At D42, two (1.7%) had DSA against class I (MFI of 1300 and 2058) and 12 (10.1%) against Class II molecules (MFI ranging from 263 to 9000). Specificities and MFIs were not modified by vaccination. Only one patient had de novo DSA against 2 different class II antigens but at very low titers (MFI < positivity threshold) without clinical consequences and remained unchanged after the second injection.

Clinical episodes suggestive of influenza A infection occurred in 3 patients, one at day 3 (virologically confirmed) and two at day 23 and day 27 (not virologically confirmed).

4. Discussion

This prospective study showed that two doses of the non-adjuvanted H1N1pdm2009 vaccine induce a significant, though relatively low, immune response in renal transplant adults with triple immunosuppressive regimen. The seroprotection rate increased significantly from 19% to 53% and 60% after one and two doses respectively and was maintained until 6 months post-vaccination. Similarly, the seroconversion rate increased significantly to 24% and 32% with a 3.7 and 4.6 fold GMT rise after the first and second injections, respectively. Age was the only factor negatively influencing the rate of response.

Our results after the first dose are below the EMEA criteria, as reported after a single dose of a non-adjuvanted vaccine in 151 renal transplant recipients with 44% seroprotection and 32% seroconversion rates [17], but are nevertheless higher than the ones reported in a small study of 18 kidney transplants recipients showing 11% and 6% seroprotection and seroconversion rates after one dose [18].

The benefit of a second dose to increase immunogenicity has already been demonstrated for an adjuvanted H1N1pdm2009 vaccine in patients with hematological malignancies but not in renal transplant recipients [19,32]. Our results evidenced the ability of two dose regimen of un-adjuvanted pandemic vaccine to improve vaccine responses in kidney transplant adults in contrast with earlier studies of seasonal and pandemic vaccine in the same settings [13,18,33,34]. Differences might be explained by a lower response to the trivalent than to the monovalent vaccine, as recently suggested in healthy subjects in whom a monovalent H1N1pdm2009 vaccine immunogenicity was slightly higher than the trivalent one, though both reached protection criteria [35]. Importantly the two doses of non-adjuvanted vaccine used here yielded comparable immunogenicity to that reported after a single dose adjuvanted pandemic vaccine where seroconversion rates ranged between 32 and 44% [19–21], except for one study with 75% in kidney transplant adults [23].

The seroprotection and seroconversion rate and GMT we report after two doses remained stable at 6 months, suggesting this regimen might be superior to a single adjuvanted vaccine injection showing the decline from 82% down to 22.5% at one year [23,36]. To our knowledge, this is the first demonstration that two injections of a non-adjuvanted vaccine are capable to induce a stable immune memory at 6 months in renal transplanted adults.

The humoral immunogenicity we report, though improved by the second dose, remains much lower than the seroprotection rates of 92% and 80%, respectively, reported with two doses of the same non-adjuvanted monovalent H1N1pdm2009 vaccine in other immunocompromised populations such as HIV-infected or SLE patients [37,38]. This suggests that the level of immunosuppression in kidney transplant recipients is much stronger than in those two groups of immunosuppressed patients. Most of our patients received tacrolimus and MMF, drugs previously associated with a profound reduction of immune response to influenza vaccines [7,13,17,39,40]. Accordingly, the low humoral immunogenicity in kidney transplant recipients is in accordance with the very weak T cell immunogenicity, though no correlations were observed between two sets of responses, as previously reported [9,39,41].

The time interval from renal transplantation plays a role since patients transplanted less than 87 months before had a lower antibody response [40]. This could also explain our results, since 75% of patients had been transplanted for less than 71 months. Of note, contrasting with other studies [19,40], the time interval since transplantation and other factors as gender and treatment did not play a role. Age was the only factor significantly influencing the vaccination outcome in our study, suggesting the major therapeutic immunosuppression administered in these transplant recipients is provoking an accelerated immune senescence. Importantly, evaluation of influenza vaccines in immunosuppressed populations questions the criteria to be used. These criteria have been defined by the European Union Committee for Medicinal Products for Human Use (CHMP) for immunocompetent adults below 60 years-old (seroprotection >70%, seroconversion >40% and geometric mean fold rise >2.5) with at least one to be reached for the seasonal vaccine, but the three criteria for the pandemic vaccine. In our study, these pandemic criteria were not achieved despite the second dose, as seroprotection was only 60% at D42. In contrast, the three CHMP requirements (seroprotection >60%, seroconversion >30%, geometric mean fold rise >2.0) defined for the elderly (>60 years) were met after our two doses of the non-adjuvanted vaccine. Regulatory requirements for influenza vaccines close to the ones followed for aging might be required in transplant recipients, as in other immunocompromised patients.

In earlier rare reports, influenza infection has been associated with onset of graft rejection [2,42–44], raising the theoretical question of an increased risk of acute rejection after vaccination and subsequent immune stimulation [11,45]. In our study, no decline of renal function and no acute rejection related to vaccination were reported. The baseline proportion of anti-HLA antibodies specificities or MFIs of DSAs remained unchanged after two vaccinations, except in one patient, though at a very low titer, as previously reported for seasonal unadjuvanted vaccine [41], but contrasting with a study using a H1N1 pandemic adjuvanted vaccine [19].

Our study has some limitations. The trial did not include a healthy control group. However, several studies have assessed this new non-adjuvanted monovalent H1N1pdm2009 vaccine in healthy populations and populations of interest. In addition, it was not acceptable to compare in a randomized trial one vs. two doses of vaccine in renal transplant patients since French guidelines recommended two injections in immunocompromised individuals [14].

The study was not designed to evaluate clinical protection against influenza and the clinical consequences of the H1N1pdm2009 influenza have been more limited than previously expected.

In conclusion, our results confirm that immunizing transplant patients against influenza remains a challenge and requires new strategies. This study demonstrates that the administration of two doses of a non-adjuvanted influenza vaccine in renal transplant patients is safe and induces a significant seroprotection, not strong enough to meet the European or US requirements for pandemic vaccine in adults below 60 years, but comparable to seroprotection usually observed in the non immunosuppressed elderly, and acceptable for a seasonal influenza vaccine. This suggests that specific regulatory requirements should be developed for immunocompromised patients and re-inforces the value of vaccination against seasonal influenza in transplant patients.

Financial support

This study was supported by a grant of the Programme de Recherche A(H1N1) coordinated by the "Institut de Microbiologie et Maladies Infectieuses", and partly by a grant from Inserm and from Sanofi Pasteur.

Conflict of interest statement

The authors of this manuscript have no conflicts of interest.

Appendix A.

List of the Inserm C09-32 TRANSFLUVAC study group:

B. Barrou, F. Thibault, B. Autran, N. Le Corre, V. Gazon, L. Avron, M. Barrois, A. Samri, M-H. Fievet, A. Boudifa, I. Theodorou (Hôpital Pitié Salpêtrière, Paris); E. Morelon, S. Daoud, Ch. Malcus, C. Dollinger, R. Sanogo, C. Pivot (Hôpital Edouard Herriot, Lyon); C. Pouteil Noble, R. Cahen, A-C. Du Besset, E. Villar, M. Rolland, C. Roufiol (Centre Hospitalier Lyon Sud, Lyon); D. Bottigioli, B. Guichard, Ch. Ravot, C. Goujon (Unité de Recherche Clinique en Immunologie, Lyon Sud); V. Dubois (Laboratoire HLA, EFS Rhône Alpes, Lyon); S. Boy, A. Puech (Inserm, Paris); J-P. Aboulker, A. Arulananthan, I. Charreau, P. Lacoste, V. Meiffrédy, M. Resch (Methodology and coordinating center, INSERM SC10, Villejuif).

References

[1] Wendt CH. Community respiratory viruses: organ transplant recipients. Am J Med 1997;102(March (3A)):31–6.
[2] Vilchez RA, McCurry K, Dauber J, Lacono A, Griffith B, Fung J, et al. Influenza virus infection in adult solid organ transplant recipients. Am J Transplant 2002;2(March (3)):287–91.
[3] Nichols WG, Guthrie KA, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis 2004;39(November (9)):1300–6.
[4] Guidelines for vaccination of solid organ transplant candidates and recipients. Am J Transplant 2004;4(November (Suppl. 10)):160–3.
[5] Avery RK, Michaels M. Update on immunizations in solid organ transplant recipients: what clinicians need to know. Am J Transplant 2008;8(January (1)):9–14.
[6] Ballout A, Goffin E, Yombi JC, Vandercam B. Vaccinations for adult solid organ transplant recipient: current recommendations. Transplant Proc 2005;37(July–August (6)):2826–7.
[7] Smith KG, Isbel NM, Catton MG, Leydon JA, Becker GJ, Walker RG. Suppression of the humoral immune response by mycophenolate mofetil. Nephrol Dial Transplant 1998;13(January (1)):160–4.
[8] Sanchez-Fructuoso AI, Prats D, Naranjo P, Fernandez-Perez C, Gonzalez MJ, Mariano A, et al. Influenza virus immunization effectivity in kidney transplant patients subjected to two different triple-drug therapy immunosuppression protocols: mycophenolate versus azathioprine. Transplantation 2000;69(February (3)):436–9.
[9] Ballet C, Roussey-Kesler G, Aubin JT, Brouard S, Giral M, Miqueu P, et al. Humoral and cellular responses to influenza vaccination in human recipients naturally tolerant to a kidney allograft. Am J Transplant 2006;6(November (11)):2796–801.
[10] Birdwell KA, Ikizler MR, Sannella EC, Wang L, Byrne DW, Ikizler TA, et al. Decreased antibody response to influenza vaccination in kidney transplant recipients: a prospective cohort study. Am J Kidney Dis 2009;54(July (1)):112–21.
[11] Briggs WA, Rozek RJ, Migdal SD, Shillis JL, Brackett RG, Brandon FB, et al. Influenza vaccination in kidney transplant recipients: cellular and humoral immune responses. Ann Intern Med 1980;92(April (4)):471–7.
[12] Keshtkar-Jahromi M, Argani H, Rahnavardi M, Mirchi E, Atabak S, Tara SA, et al. Antibody response to influenza immunization in kidney transplant recipients receiving either azathioprine or mycophenolate: a controlled trial. Am J Nephrol 2008;28(4):654–60.
[13] Scharpé J, Evenepoel P, Maes B, Bammens B, Claes K, Osterhaus AD, et al. Influenza vaccination is efficacious and safe in renal transplant recipients. Am J Transplant 2008;8(February (2)):332–7.
[14] Haut Conseil de la santé publique. Actualisation de l'avis relatif aux recommandations sur l'utilisation des vaccins pandémiques dirigés contre le virus grippal A(H1N1)v. Avis du 28 octobre 2009. Available at http://www.hcsp.fr/docspdf/avisrapports/hcspa20091028_H1N1.pdf (accessed 27.06.12).
[15] Kumar D, Morris MI, Kotton CN, Fischer SA, Michaels MG, Allen U, et al. Guidance on novel influenza A/H1N1 in solid organ transplant recipients. Am J Transplant 2010;10(January (1)):18–25.
[16] Long CB, Ramos I, Rastogi D, Manwani D, Janow G, Del Rio M, et al. Humoral and cell-mediated immune responses to monovalent 2009 influenza A/H1N1 and seasonal trivalent influenza vaccines in high-risk children. J Pediatr 2012;160(January (1)):74–81.
[17] Mulley WR, Visvanathan K, Hurt AC, Brown FG, Polkinghorne KR, Mastorakos T, et al. Mycophenolate and lower graft function reduce the seroresponse of kidney transplant recipients to pandemic H1N1 vaccination. Kidney Int 2012;82(July (2)):212–9.
[18] Beaudreuil S, Krivine A, Hebibi H, Ducot B, Mazet AA, Taouffik Y, et al. Efficacy and safety of the H1N1 monovalent vaccine in renal-transplant recipients and dialysis patients. Hum Vaccin 2011;7(August (8)):868–73.
[19] Brakemeier S, Schweiger B, Lachmann N, Glander P, Schonemann C, Diekmann F, et al. Immune response to an adjuvanted influenza A H1N1 vaccine (Pandemrix®) in renal transplant recipients. Nephrol Dial Transplant 2012;27(January (1)):423–8.
[20] Broeders NE, Hombrouck A, Lemy A, Wissing KM, Racape J, Gastaldello K, et al. Influenza A/H1N1 vaccine in patients treated by kidney transplant or dialysis: a cohort study. Clin J Am Soc Nephrol 2011;6(November (11)):2573–8.
[21] Crespo M, Collado S, Mir M, Cao H, Barbosa F, Serra C, et al. Efficacy of influenza A H1N1/2009 vaccine in hemodialysis and kidney transplant patients. Clin J Am Soc Nephrol 2011;6(September (9)):2208–14.
[22] Kelen K, Ferenczi D, Jankovics I, Varga M, Molnar MZ, Sallay P, et al. H1N1 vaccination in pediatric renal transplant patients. Transplant Proc 2011;43(May (4)):1244–6.
[23] Cordero E, Perez-Ordonez A, Aydillo TA, Torre-Cisneros J, Gavalda J, Lara R, et al. Therapy with m-TOR inhibitors decreases the response to the pandemic influenza A H1N1 vaccine in solid organ transplant recipients. Am J Transplant 2011;11(October (10)):2205–13.
[24] Felldin M, Studahl M, Svennerholm B, Friman V. The antibody response to pandemic H1N1 2009 influenza vaccine in adult organ transplant patients. Transpl Int 2012;25(February (2)):166–71.
[25] European committee for proprietary medicinal products. Guideline on dossier structure and content for pandemic influenza vaccine marketing authorization application (CPMP/VEG/4717/03). European agency for the evaluation of medicinal products; 2004.
[26] European Committee for Proprietary Medicinal Products. Guideline on influenza vaccine prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context (EMEA/CHMP/VWP/263499/2006). European Agency for the Evaluation of Medicinal Products; January 2007.
[27] FDA Guidance for Industry. Clinical data needed to support the licensure of pandemic influenza vaccines. US Food and Drug Administration; May 2007. Available at http://www.fda.gov/cber/gdlns/panfluvac.htm (accessed 27.06.12).
[28] World Health Organization. Availability of a candidate reassortant vaccine virus for the novel Influenza A (H1N1) vaccine development X-179A. Available at http://www.who.int/csr/resources/publications/swineflu/candidates_X-179a/en/index.html (accessed 5.02.11).
[29] Kendal AP, Skehel JJ, Pereira MS. Concepts and procedures for laboratory-based influenza surveillance. Atlanta, GA: US Department of Health and Human Services, Public Health Service Centers for Disease Control; 1982.

[30] Papagno L, Alter G, Assoumou L, Murphy RL, Garcia F, Clotet B, et al. Comprehensive analysis of virus-specific T-cells provides clues for the failure of therapeutic immunization with ALVAC-HIV vaccine. AIDS 2011;25(January (1)):27–36.
[31] EMEA. Note for guidance on harmonization of requirements for influenza vaccines. 12 March 1997. CPMP/BWP/214/96. Available at http://www.emea.europa.eu/pdfs/human/bwp/021496en.pdf (accessed 5.02.11).
[32] de Lavallade H, Garland P, Sekine T, Hoschler K, Marin D, Stringaris K, et al. Repeated vaccination is required to optimize seroprotection against H1N1 in the immunocompromised host. Haematologica 2011;96(February (2)):307–14.
[33] Blumberg EA, Albano C, Pruett T, Isaacs R, Fitzpatrick J, Bergin J, et al. The immunogenicity of influenza virus vaccine in solid organ transplant recipients. Clin Infect Dis 1996;22(February (2)):295–302.
[34] Versluis DJ, Beyer WE, Masurel N, Wenting GJ, Weimar W. Impairment of the immune response to influenza vaccination in renal transplant recipients by cyclosporine, but not azathioprine. Transplantation 1986;42(October (4)):376–9.
[35] Van der Vliet D, Pepin S, Lambert M, Fauchoux N, Donazzolo Y, Dupuy M, et al. Similar immunogenicity of the A/H1N1 2009 pandemic influenza strain when used as a monovalent or a trivalent vaccine. Hum Vaccin 2010;6(October (10)):823–8.
[36] Cordero E, Aydillo TA, Perez-Ordonez A, Torre-Cisneros J, Lara R, Segura C, et al. Deficient long-term response to pandemic vaccine results in an insufficient antibody response to seasonal influenza vaccination in solid organ transplant recipients. Transplantation 2012;93(April (8)):847–54.
[37] Mathian A, Devilliers H, Krivine A, Costedoat-Chalumeau N, Haroche J, Huong DB, et al. Factors influencing the efficacy of two injections of a pandemic 2009 influenza A (H1N1) non-adjuvanted vaccine in systemic lupus erythematosus. Arthritis Rheum 2011;63(November (11)):3502–11.
[38] Launay O, Desaint C, Durier C, Loulergue P, Duval X, Jacomet C, et al. Safety and immunogenicity of a monovalent 2009 influenza A/H1N1v vaccine adjuvanted with AS03A or unadjuvanted in HIV-infected adults: a randomized, controlled trial. J Infect Dis 2011;204(July (11)):124–34.
[39] Lindemann M, Witzke O, Lutkes P, Fiedler M, Kreuzfelder E, Philipp T, et al. ELISpot assay as a sensitive tool to detect cellular immunity following influenza vaccination in kidney transplant recipients. Clin Immunol 2006;120(September (3)):342–8.
[40] Salles MJ, Sens YA, Boas LS, Machado CM. Influenza virus vaccination in kidney transplant recipients: serum antibody response to different immunosuppressive drugs. Clin Transplant 2010;24(January–February (1)):E17–23.
[41] Candon S, Thervet E, Lebon P, Suberbielle C, Zuber J, Lima C, et al. Humoral and cellular immune responses after influenza vaccination in kidney transplant recipients. Am J Transplant 2009;9(October (10)):2346–54.
[42] Briggs JD, Timbury MC, Paton AM, Bell PR. Viral infection and renal transplant rejection. Br Med J 1972;4(December (5839)):520–2.
[43] Gabriel R, Selwyn S, Brown D, Crossland JM, Loughridge LW, Morgan MN, et al. Virus infections and acute renal transplant rejection. Nephron 1976;16(4):282–6.
[44] Lopez C, Simmons RL, Mauer SM, Najarian JS, Good RA, Gentry S. Association of renal allograft rejection with virus infections. Am J Med 1974;56(March (3)):280–9.
[45] Kimball P, Verbeke S, Flattery M, Rhodes C, Tolman D. Influenza vaccination does not promote cellular or humoral activation among heart transplant recipients. Transplantation 2000;69(June (11)):2449–51.