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Higher Memory Responses in HIV-Infected and Kidney Transplanted Patients than in Healthy Subjects following Priming with the Pandemic Vaccine

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Abstract

Background: Memory responses require immune competence. We assessed the influence of priming with AS03-adjuvanted pandemic vaccine (Pandemrix®) on memory responses of HIV patients, kidney recipients (SOT) and healthy controls (HC).

Method: Participants (HIV: 197, SOT: 53; HC: 156) were enrolled in a prospective study and 390/406 (96%) completed it. All had been primed in 2009/2010 with 1 (HC) or 2 (patients) doses of Pandemrix®, and were boosted with the 2010/2011 seasonal influenza vaccine. Geometric mean titres and seroprotection rates were measured 12 months after priming and 4 weeks after boosting. Primary and memory responses were directly compared in 191 participants (HCW: 69, HIV: 71, SOT: 51) followed during 2 consecutive seasons.

Results: Most participants (HC: 77.8%, HIV: 77.6%, SOT: 66%) remained seroprotected at 12 months post-priming. Persisting A/09/H1N1 titers were high in HIV (100.2) and HC (120.1), but lower in SOT (61.4) patients. Memory responses reached higher titers in HIV (507.8) than in HC (253.5) and SOT (136.9) patients. Increasing age and lack of HAART reduced persisting and memory responses, mainly influenced by residual antibody titers. Comparing 2009/2010 and 2010/2011 titers in 191 participants followed for 2 seasons indicated lower post-2010/2011 titers in HC (240.2 vs 313.9), but higher titers in HIV (435.7 vs 338.0) and SOT (136 vs 90.3) patients.

Conclusions: Priming with 2 doses of Pandemrix® elicited persistent antibody responses and even stronger memory responses to non-adjuvanted seasonal vaccine in HIV patients than 1 dose in healthy subjects. Adjuvanted influenza vaccines may improve memory responses of immunocompromised patients.

Trial Registration: ClinicalTrials.gov NCT01022905

Introduction

Immunosuppressed patients are at higher risks of influenza complications. This was evidenced by reports of disease outcome in solid organ transplant (SOT) recipients infected by the pandemic influenza A/09/H1N1 strain [1–6]. A similar risk exists in HIV infected individuals with advanced disease and low CD4 cell count but not in HAART-treated patients [7–11]. Immunosuppressed patients have a general trend toward impaired antibody responses to non-adjuvanted vaccines [6]. Short-term antibody responses were indeed lower following 1 or 2

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doses of non-adjuvanted influenza A/09/H1N1 vaccines in HIV-infected [12,13] and solid organ transplant (SOT) patients [13–15]. The extent to which adjuvanted vaccines may improve responses is thus of central interest. In HIV-infected patients, a single dose of the AS03-adjuvanted pandemic vaccine (Pandemrix®) elicited higher responses than non-adjuvanted monovalent vaccines [16]. Four weeks after 1 dose of Pandemrix®, sero-responses remained lower than in controls [17,18] reaching similar titers after 2 doses [19–21]. Seroresponses remained lower in SOT recipients even after 2 doses of Pandemrix® [19,22,23], reflecting a more profound impact of immunosuppression on vaccine responses.

How immunosuppression affects memory responses is less well defined. In HIV-infected patients, impaired B and T cell functions cause dysfunctional germinal center interactions [24] and result in a progressive loss of B-cell memory despite antiretroviral therapy [25–27]. Accordingly, most HAART-treated HIV-infected adults reached a hemagglutination inhibition titer (HAI) ≥1/40 four weeks after immunization with non-adjuvanted A/09/H1N1 vaccines but only 28% remained above this threshold at 6 months [28]. How the immunosuppression of SOT patients affects memory responses is less well described.

In 2009/2010, we had followed 760 immunocompromised and 133 healthy adults immunized with 1 (healthy) or 2 (patients) doses of Pandemrix®. Four weeks after immunization, we observed similar responses in HIV-infected individuals after 2 doses as in healthy adults after 1 dose [20], and lower seroresponses in SOT recipients despite 2 immunizations [23]. To define how adjuvanted vaccines would influence antibody persistence and memory responses, we assessed the impact of 2009/2010 priming with Pandemrix® on antibody persistence and memory responses elicited in 2010/2011 by one dose of a non-adjuvanted trivalent inactivated seasonal vaccine including the same influenza A/09/ H1N1 strain.

Patients and Methods

Subjects

This prospective, multisite, open-label study recruited 406 subjects in November 2010:197 HIV-infected patients, 53 SOT (kidney) recipients and 156 healthy controls (HC) (Figure S1). Inclusion criteria included age above 18 years and having received 1 (controls) or 2 (patients) doses of AS03-adjuvanted pandemic influenza vaccine in 2009/2010. Among these 406 subjects, 191 subjects (69 HIV-infected patients, 51 kidney transplant recipients and 71 controls) had already been enrolled in 2009/2010 and were followed for 2 seasons. The latter are referred to as the 2009–2010–2011 cohort. The study was approved by the institutional review board (ID: CER-09-234), registered on ClinicalTrials.gov prior to patient enrolment (ID: NCT01022905) and conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice, and Swiss regulatory requirements. Written informed consent was obtained prior to inclusion in 2009 and in 2010.

The protocol for this trial is available as supporting information, see Protocol S1.

Vaccines and Immunizations

In 2010/2011, patients and controls received one intramuscular dose of a non-adjuvanted trivalent split-virus influenza vaccine containing 15 μg of A/California/07/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008. Most patients (>98% in each group) received Mutagrip® (SanofiPasteurMSD). In 2009/2010, all participants had received 1 (healthy controls) or 2 doses (HIV-infected or SOT patients) of AS03-adjuvanted split-virus influenza A/09/H1N1 vaccine (Pandemrix®, GlaxoSmithKline) at a 4 weeks interval, according to official Swiss recommendations. Each dose of Pandemrix® contained 3.75 μg of A/09/H1N1 antigen emulsified in squalene, DL-a-tocopherol and polysorbate 80.

Data Collection

Medical information was obtained through a detailed medical history and completed through the patient’s records. Blood was collected on the day of the first dose and 4 weeks after immunization. Sera were prepared and stored at -20°C until used.

Haemagglutination Inhibition (HAI) Assay

HAI assays were performed as described [29]. Sera were subjected to 2-fold serial dilutions prior to incubation with 4 or 8 HA units of pandemic influenza A/California/07/09 (H1N1) and seasonal A/Perth/09 (H3N2) virus, respectively. Results were expressed as the reciprocal of the highest dilution showing a positive HAI. Negative samples were assigned a titre of 1:4 and individual values were log transformed to calculate the geometric mean antibody titres (GMT).

The three co-primary immunogenicity end-points were: 1) The GMTs based on individual HAI titres, 2) the proportion of seroprotected subjects (defined as a post-vaccination HAI-titre of ≥1:40) and 3) the proportion of subjects with a seroresponse (defined as a post-vaccination HAI-titre of ≥1:40 and a fold increase in GMT of ≥4 between pre-vaccination and post-vaccination HAI- titres).

Safety Monitoring

Safety endpoints were divided into local inflammation (injection-site pain, erythema and swelling) and systemic reactions (fever, fatigue, headaches and anorexia). They were self-recorded for 7 days after each dose. Serious adverse events (SAE) were actively searched for and followed up until their resolution; recording stopped on February 28, 2010, at study termination. In HIV-infected patients, HIV-RNA levels were measured 4 weeks after immunization to detect potential increases in HIV-RNA levels. Quantitative plasma HIV-1 RNA was measured on a Roche COBAS TaqMan HIV-1 test version 2.0 (Roche Diagnostics, Basel, Switzerland). Transplant patients were closely followed and instructed to contact the study investigator in case of adverse events.

Statistical Analysis

Due to the lack of data concerning the long-term immunogenicity of Pandemrix® at time of study design, sample size was based on recruitment capacity. GMTs were given with 95% confidence interval. The reverse cumulative distributions were presented. The comparison of titres between individual strata was assessed by means of the Kruskal-Wallis test. For longitudinal comparisons, the Wilcoxon test for paired data was used (for instance baseline vs post-baseline titers from same patients). Comparisons of proportions were performed by using Chi-squared tests (or Fisher exact test if frequencies were less than 5) and McNemar tests for paired data. Multivariate regression models were constructed to investigate the association between specific independent variables and post-vaccination antibody titres. The selection of factors introduced in the models was determined a priori. Data were logarithmically transformed prior to analysis. The parameter of the linear model indicated the variation on the log-transformed titre and we interpreted the meaning of the
regression parameters as the percentage increase of the titre per
given unit (for continuous factors) or compared to the reference
category (for categorical factors). A logistic regression was
performed to assess the association of the gender on the risk of
pain adjusted on the group of patients. An odds ratio greater than
1 was interpreted as an increase of risk in women compared to
men. The significance level was defined as 0.05. Data were
analysed by using S-PLUS 8.0, Insightful Corp., Seattle, WA
(USA).

Results

Characteristics of Immunosuppressed Patients and
Healthy Subjects

The clinical characteristics of the 197 HIV-infected patients, 53
SOT recipients and 156 healthy controls recruited in 2010/2011
are described in Table 1. All had been immunized with Pandemrix® in 2009/2010. HCs included a higher percentage of
females and were younger. Most participants were Caucasians
(82%) and had been immunized against seasonal influenza in
2009. HIV-infected patients were characterized by their CDC
category, CD4 T cell count and nadir (Table 1). Most were
found to be treated with antiretroviral drugs. Kidney transplant recipients had
reduced creatinine clearance (median 52, IQR 38–60). Most were
on maintenance mycophenolate acid, tacrolimus and low dose
steroids (Table 1). All had been transplanted more than one year ago
(median 9.1 years, IQR 4.8–13.4, minimum 1.3 year). Two
had been treated for a rejection episode within 12 months (40 and
351 days before immunization).

All participants but one HC (newly recognized pregnancy)
received a single dose of a non-adjuvanted trivalent inactivated
2010–2011 seasonal influenza vaccine, mostly Mutagrip®, allowing
to directly compare their 2010–2011 responses despite
differences in their 2009 immunization history. Seven HIV-
positive patients and 1 SOT recipient remained unreachable. One
SOT patient died of a non-vaccine related event. Six HCs
withdrew consent to the 2nd blood sample. Altogether, 190/197
(96.4%) of HIV patients, 51/53 (96.2%) of SOT recipients and
149/156 (95.5%) healthy subjects were included in the 2010/2011
analysis of vaccine responses (Figure S1).

Safety

Immunization was generally well tolerated (Table S1). Local
pain was more frequently reported by HCs (59.7% (CI95% 51.4;
67.7)) than HIV (42.1 (CI95% 35; 49.5)) or SOT (35.3 (CI95%
22.4; 49.9), p<0.001) patients. Women reported pain more
frequently than men (OR adjusted on group: 2.06, CI95% 1.34–
3.17, p<0.001) and, despite the higher proportion of women
among HCs, the group effects remained significant: OR = 0.62
(CI95% 0.39–0.93, p = 0.04) for HIV compared to HCs and
OR = 0.43 (CI95% 0.22–0.85, p = 0.02) for kidney recipients
compared to HCs. Systemic reactions were rare and evenly
distributed. Four weeks after immunization, HIV-RNA were below
detection levels (172/197, 93%) or low (median 136 copies/mL,
IQR 58–3048). SOT patients did not require any change of their
immunosuppression regimen. Two severe adverse events (1 death,
1 amputation) were considered as unrelated to immunization.

Antibody Persistence 12 Months after Monovalent AS03-
adjuvanted Influenza A/09/H1N1 Immunization

Antibody titers persisting 12 months after Pandemrix®
immunization were assessed by HAI in 153/156 (98.0%) HCs, 196/197
(99.5%) HIV patients and all (53) kidney recipients. Most
participants (HIV: 77.6%; HC: 77.8%; SOT 66%) remained
seroprotected. Residual HAI-GMTs (pre-2010) were as high in
HIV patients (100.2, CI95% 86.3–116.2) as in HCs (120.1, CI95%
99.7–144.6). Despite 2 doses of Pandemrix®, titers were lower in
SOT recipients (61.4, CI95% 44.0–85.7) than in HIV patients or
controls (Table 2A and Figure 1A-B).

Regression analyses indicated that increasing age significantly
lowered antibody persistence, reflected by lower pre-2010 HAI-
GMTs (Table 3) and seroprotection rates (HC >60 years: 36.4% (CI95%
10.9-69.2) vs 81.0% (CI95% 73.6-87.1) for subjects ≤60
years, p = 0.003; HIV >60 years: 63.3% (CI95% 43.9-80.1) vs 80.1%
(CI95% 73.2-85.9) for subjects ≤60 years, p = 0.07). Multivariate
analyses (Table 4) confirmed that each additional 10 years reduced
pre-2010 HAI-GMTs, although not significantly in HIV patients.
Antibodies persisted at higher titers in HIV patients with CD4 counts
>350/mm3 or HAART treated (Table 4). The immunosuppression
regimens were too homogenous to lead to significant drug-specific
influences (Table 3 and 4). Gender and 2009/2010 seasonal
influenza immunization exerted no impact (not shown).

Memory Responses to Influenza A/09/H1N1
Immunization during the 2010/2011 Season

Four weeks after boosting with 1 dose of non-adjuvanted
inactivated 2010–2011 trivalent vaccine including again the
influenza A/09/H1N1 strain, HAI-GMTs had significantly
increased in each group (Figure 1A-C). HCs raised strong
titers (fold increase 2.07 (CI95% 1.81–2.37), p<0.001) and
reached higher seroprotection rates (95.8 (CI95% 91.2-98.5) vs
77.8 (CI95% 70.4-84.1), p<0.001) compared to baseline
(Figure 1A and Table 2A). Antibody responses were even
stronger in HIV patients (fold increase 4.99 (4.22; 5.91,
p<0.001), their post-immunization HAI titers reaching 2-fold
higher levels than those of HCs (p<0.001), and a remarkable
98.4% (CI95% 95.4-99.7) seroprotection rate. One dose of
seasonal 2010/2011 vaccine also increased the HAI titers of
SOT recipients (fold increase 2.83 (1.64; 3.19), p<0.001).
However, HAI-titers and seroprotection rates remained lower
than in controls (Table 2A). Despite adjustment for age, HAITiters also remained 65.7% lower in SOT than in HIV patients
(p<0.001) similarly primed in 2009 with 2 doses of Pandemrix®,
reflecting their lower immune competence. Serocconversion
rates, less informative when baseline antibodies are high, were
significantly higher in HIV patients (Table 2A) despite similar
pre-2010 HAI-GMTs, reflecting superior memory responses.

Increasing age significantly impacted the post-2010 antibody
responses: each additional 10 years independently reduced post-
immunization titers by 20% (HC), 22% (HIV) or 29% (SOT
(Table 4). Antibody responses appeared paradoxically better in
HIV patients with more advanced disease (Table 3), but
multivariate analyses identified HAART as independently associ-
ated with higher vaccine responses (Table 4). Memory responses
were lower in MMF-treated patients (-2%), without reaching
statistical significance.

The strongest determinant of the 2010/2011 antibody responses
was the residual (pre-2010) antibody titer. This influence was
positive: post-2010 titers were significantly higher (p<0.001) when
persisting antibodies were above as compared to below the group
median (Table 3). Adding persisting antibodies in the regression
analyses indicated that they increased post-2010/2011 titers by
97% (HC), 69.6% (HIV) and 137.6% (SOT) (not shown). As post-
2010/2011 HAI-GMT were significantly higher in HIV patients
compared to HCs after controlling for age, gender and pre-2010
titers (p<0.001), we focused subsequent analyses on participants
followed during 2 seasons.
Influence of 2009/2010 Immunization on 2010/2011 Memory Responses to Influenza A/09/H1N1

HAI titers elicited in 2009/2010 (post-2009), persisting in 2010/2011 (pre-2010) and reactivated in 2010/2011 (post-2010) were compared for the 191 patients of the 2009/2010/2011 cohort (HC: 69, HIV: 71, SOT: 51) (Table 2B and Figure 1E).

As described [20,23], post 2009/2010 HAI-GMTs were similar in HIV individuals and HCs, and lower in SOT recipients (Table 2B and Figure 1E). HAI-GMTs declined during the following year and increased after the 2010/2011 immunization. In HCs, post-2010/2011 HAI titers remained significantly lower than in 2009/2010 (240.2 vs 313.9, p<0.001) (Table 2B and Figure 1E). In contrast, higher HAI-GMTs were observed in HIV patients following boosting with 1 dose of seasonal 2010/2011 vaccine as compared to priming with 2 doses of Pandemrix® (HAI: 435.7 vs 330.0, p = 0.03; post-2010/post-2009 GMT ratio: 1.365 (1.034; 1.803), p = 0.03). A similar trend towards higher post-2010/2011 responses was observed in SOT recipients (HAI: 136.0 vs 90.3, p = 0.20; GMT ratio 1.502 (0.992; 2.275), p = 0.055). These better 2010/2011 than 2009/2010 responses were not ascribed to higher immune competence: changes in the medical conditions (7 patients) or antiretroviral therapy (13 changes, including 8 changes of drug class) of HIV patients were not considered as increasing immune competence, and immunosuppression intensity had been reduced in 8 but increased in 8 others SOT recipients.

### Table 1. Baseline characteristics.

|                      | Total | Controls | HIV | Kidney recipient | p value* |
|----------------------|-------|----------|-----|------------------|----------|
| Women N (%)          | 156   | 106      | 62  | 20               | 37.70%   |
| Median age, years (IQR) | 42.9 (33.8–51.7) | 47.6 (40.9–55.3) | 61.6 (55.6–69.7) | <0.001 |
| <40 yrs N (%)        | 61    | 44       | 3   | 5.70%            | <0.001   |
| 40–60 yrs N (%)      | 84    | 123      | 21  | 39.60%           |          |
| >60 yrs N (%)        | 11    | 30       | 29  | 54.70%           |          |
| Seasonal influenza 2009 N (%) | 148 | 183      | 46  | 86.80%           | 0.14     |
| Ethnicity, N (%)     |       |          |     |                  |          |
| Caucasian           | 148   | 129      | 47  | 88.7%            |          |
| Afri. Sub-saharian   | 4     | 43       | 3   | 5.7%             |          |
| Others               | 4     | 25       | 3   | 5.7%             |          |
| CDC category A, N (%)|       |          |     |                  |          |
| B, N (%)             | 51    | 30.50%   | 84  | 42.60%           |          |
| C, N (%)             | 56    | 28.60%   | 4   | 2.6%             |          |
| CDC4 Baseline, cells/mm3 |       |          |     |                  |          |
| Median (IQR)         | 615   | 458–769  |     |                  |          |
| Nadir, cells/mm3     |       |          |     |                  |          |
| Median (IQR)         | 172   | 50–288   |     |                  |          |
| Time since transplant, years Median (IQR) | 9.1 (4.8–13.4) | 9.1 (4.8–13.4) | 9.1 (4.8–13.4) |          |
| Rejection episodes N(%) | 51   | 96.20%   | 2   | 3.80%            |          |
| previous 12 months N(%) | 2    | 3.80%   | 11  | 20.80%           |          |
| Immunosuppressive treatment |       |          |     |                  |          |
| Cyclosporin, N(%)    | 11    | 20.80%   |     |                  |          |
| Tacrolimus, N (%)    | 37    | 69.80%   |     |                  |          |
| Evero- or Sirolimus, N (%) | 2 | 3.80%   |     |                  |          |
| MMF or EC-MPA, N (%) | 35    | 66.00%   |     |                  |          |
| Azathioprine, N (%)  | 8     | 15.10%   |     |                  |          |
| Others, N(%)         | 1     | 1.90%    |     |                  |          |
| Oral steroids N(%)   | 28    | 52.80%   |     |                  |          |
| Low oral dose (<10 mg) N(%) | 25 | 89.30%   |     |                  |          |

*IQR: interquartile range; PI: protease inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; ART: antiretroviral therapy; HAART: highly active antiretroviral therapy; MMF: mycophenolate mofetil; EC-MPA: enteric-coated mycophenolic acid.

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To compare the immune competence of HIV and HCs during the 2010/2011 season, we assessed primary antibody responses to the influenza A/09/H3N2 strain, first included in the 2010–2011 seasonal trivalent vaccine. H3N2 HAI-GMTs and seroprotection rates were low prior to immunization and increased following immunization (Table 2C and Figure 1F). H3N2 HAI-GMTs remained relatively low and responses were not significantly different among patients and controls (Table 2C and Figure 1F).

Figure 1. Distribution and kinetics of antibody titers. A–C. Blood was collected before and 4 weeks after 1 dose of non-adjuvanted 2010/2011 seasonal influenza vaccine. Antibody titers were assessed by hemagglutination inhibition (HAI). The results were expressed as the reciprocal of the highest dilution showing a positive HAI (see Methods). The vertical dotted line represents the seroprotection threshold (titer 1:40). The curves represent the distribution of individual antibody titers in each group. D–F: Geometric mean HAI titers to influenza A/09/H1N1 (D, E) and A/09/H3N2 (F) were measured before (pre-2009) and after (post-2009) immunization with 1 (controls) or 2 (patients) doses of AS03-adjuvanted pandemic vaccine, and before (pre-2010) and after (post-2010) 1 dose of non-adjuvanted 2010/2011 seasonal influenza vaccine. D: All patients followed in 2010/2011. E–F: Patients followed for 2 consecutive seasons. doi:10.1371/journal.pone.0040428.g001

Similar 2010/2011 Antibody Responses to Influenza A/09/H3N2 in HIV-infected and Healthy Subjects

To compare the immune competence of HIV and HCs during the 2010/2011 season, we assessed primary antibody responses to the influenza A/09/H3N2 strain, first included in the 2010–2011 seasonal trivalent vaccine. H3N2 HAI-GMTs and seroprotection rates were low prior to immunization and increased following immunization (Table 2C and Figure 1F). H3N2 HAI-GMTs remained relatively low and responses were not significantly different among patients and controls (Table 2C and Figure 1F).
Thus, true anamnestic responses characterized by stronger booster 2010/2011 than primary 2009/2010 responses against influenza A/09/H1N1 were observed in well-controlled HAART-treated HIV patients and moderately immunosuppressed SOT recipients, but not in healthy individuals.

Discussion

How immune competence affects memory responses and whether these may be enhanced by adjuvanted vaccines is not well known. Here we show that antibodies elicited by the AS03-adjuvanted pandemic vaccine (Pandemrix®) persisted at similarly high titers in HIV patients as in HCs up to 12 months after priming. Unexpectedly, memory responses elicited against A/09/H1N1 by non-adjuvanted trivalent 2010/2011 influenza vaccines were stronger in HIV patients primed in 2009 with 2 doses of Pandemrix® than in HCs primed with a single dose. Persisting antibody titers and memory responses were also high – although significantly lower - in SOT recipients.

The duration of vaccine-induced antibodies depends upon peak antibody production by short-lived plasma cells and plasma cell persistence. The rapid disappearance of vaccine antibodies usually observed in HIV patients, as following immunization with non-adjuvanted pandemic vaccines [28], may thus result from low peak responses and/or short-lasting antibody production. In contrast to the conclusion of Cagigi et al [24] that “even if immunisation induces a good antibody response, patients with HIV will not sustain antibody production for as long as healthy individuals because of intrinsic defects in their B cell compartment”, HAI antibodies persisted at similarly high levels in HIV patients and HCs and remained above seroprotective levels in most SOT recipients. We cannot formally demonstrate that this resulted from priming with Pandemrix®, as non-adjuvanted pandemic vaccines were unavailable in Switzerland. However, antibody persistence was better following immunization with AS03-adjuvanted than non-adjuvanted H5N1 vaccines (seroprotection rate 36% vs 6%) [30]. As younger age, antiretroviral therapy and higher CD4 counts favoured antibody persistence, shorter/weaker responses might have been observed in non-HAART treated patients.

Controls reached lower titers following boosting in 2010/2011 than priming in 2009/2010, reflecting the recruitment/activation of fewer antigen-specific B cells by 15 ug of non-adjuvanted than 3.8 ug of AS03-adjuvanted H1N1 antigens. These weaker 2010/2011 responses did not result from an inhibitory influence of higher baseline antibodies, which were positively correlated with booster responses. They are unlikely to reflect altered immune

### Table 2. Antibody persistence and memory responses to influenza A/09/H1N1 and A/09/H3N2.

| Influenza A/09/H1N1 | HAI-GMT | Seroprotection | Seroconversion |
|---------------------|---------|----------------|---------------|
|                     | N (95%CI) | N (%) | N/Total (%) |
| **A. 2010/2011 cohort** | | | |
| Controls       | Pre 2010 153 120.1 (99.7;144.6) | 119 77.8 (70.4;84.1) | |
| HIV           | Post 2010 144 253.5 (213.4;301.1) | 138 95.8 (91.2;98.5) | 66/142 46.5 (38.1;55.0) |
| Kidney recipients | Pre 2010 187 507.8 (428.7;601.4)*** | 184 98.4 (95.4;99.7) | 148/186 79.6 (73.1;85.1) *** |
| **B. 2009/2010/2011 cohort** | | | |
| Controls       | Post 2009 69 313.9 (218.6;450.8) | 60 88.2 (78.1;94.8) | |
| HIV           | Post 2009 66 338.0 (252.8;451.9) | 62 93.9 (85.2;98.3) | |
| Kidney recipients | Post 2009 68 435.7 (320.8;591.9)*** | 63 95.6 (87.6;99.1) | 49/67 73.1 (60.9;83.2) *** |
| **Influenza A/09/H3N2** | | | |
| **C. 2009/2010/2011 cohort** | | | |
| Controls       | Pre 2010 69 23.2 (16.5;32.6) | 30 43.5 (31.6;56.0) | |
| HIV           | Post 2010 67 30.2 (21.9;41.7) | 29 43.3 (31.2;56.0) | |
| Kidney recipients | Pre 2010 67 105.8 (74.5;150.3) | 54 80.6 (69.1;89.2) | 29/67 43.3 (31.2;56.0) |

HAI: hemagglutination inhibition; 2010/2011 cohort: subjects followed in 2010/2011; 2009/2010/2011 cohort: subjects followed for 2 years. Pre 2010: before 2010/2011 immunization; Post 2010:4 weeks after 2010/2011 immunization; Post 2009:4 weeks after 1.

*p <0.05, **p <0.01 and *** p <0.001 compared to controls.

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Table 3. Determinants of vaccine antibody persistence and memory responses to influenza A/09/H1N1.

|                             | N     | Pre 2010 HAI-GMT (95%CI) | Post 2010 HAI-GMT (95%CI) |
|-----------------------------|-------|--------------------------|---------------------------|
|                             |       | 166.6 (120.2;230.8)      | 335.4 (258.4;435.2)       |
| Controls Age                | <40 yrs | 61                       | 293.1 (190.8;299.5)       |
|                             | 40-60 yrs | 84                      | 46.1 (31.2;68.2)          |
|                             | >60 yrs  | 11                      | 107.0 (54.0;212.0)        |
|                             | p-value  | 0.001                    | 0.005                     |
|                             | Pre 2010 HAI-GMT | < median | 44.8 (40.3;49.9)          |
|                             | ≥ median | 79                      | 302.3 (251.7;363.0)       |
|                             | p-value  | <0.001                   | < 0.001                   |
| HIV-infected patients Age   | <40 yrs | 44                      | 98.7 (73.6;132.3)         |
|                             | 40-60 yrs | 123                     | 107.0 (54.0;212.0)        |
|                             | >60 yrs  | 11                      | 107.0 (54.0;212.0)        |
|                             | p-value  | 0.18                     | 0.13                      |
|                             | CD4 T cell count | ≥ 350 | 110.5 (94.1;129.8)       |
|                             | <350 | 29                      | 57.8 (38.8;86.0)          |
|                             | p-value  | 0.18                     | 0.01                      |
|                             | CD4 nadir | ≥ 100 | 106.5 (88.8;127.8)       |
|                             | <100 | 65                      | 88.4 (68.0;114.9)         |
|                             | p-value  | 0.29                     | 0.02                      |
|                             | CDC group A | 89                       | 93.1 (74.6;116.2)         |
|                             | B | 51                      | 130.5 (98.0;173.7)        |
|                             | C | 56                      | 88.3 (66.7;117.0)         |
|                             | p-value  | 0.12                     | 0.04                      |
|                             | HAART None | 11                     | 42.4 (28.5;63.0)          |
|                             | ART+NNRTI+PI | 20                  | 97.3 (59.4;159.4)         |
|                             | ART+NNRTI no PI | 84             | 108.3 (86.2;136.0)       |
|                             | ART+PI no NNRTI | 60            | 102.1 (77.3;134.7)       |
|                             | ART+Other | 22                    | 111.8 (74.7;167.4)        |
|                             | p-value  | 0.07                     | 0.07                      |
|                             | Pre 2010 HAI-GMT | < median | 43.0 (38.7;47.7)         |
|                             | ≥ median | 96                      | 225.7 (193.8;262.9)       |
|                             | p-value  | <0.001                   | <0.001                    |
| Kidney recipients Age      | <40 yrs | 3                       | 263.8 (27.1;2568.0)       |
|                             | 40-60 yrs | 21                      | 69.1 (41.1;116.3)         |
|                             | >60 yrs  | 29                      | 48.5 (32.5;72.4)          |
|                             | p-value  | 0.16                     | 0.63                      |
|                             | Tacrolimus No | 16                | 44.5 (22.3;88.6)          |
|                             | Yes | 37                      | 70.6 (48.7;102.3)         |
|                             | p-value  | 0.27                     | 0.20                      |
|                             | MMF No | 18                      | 52.6 (30.2;91.8)          |
|                             | or EC-MPA Yes | 35               | 66.5 (43.7;101.2)         |
|                             | p-value  | 0.78                     | 0.17                      |
|                             | Oral steroids No | 25          | 59.5 (33.4;105.9)         |
|                             | Yes | 28                      | 63.2 (43.4;920)           |
|                             | p-value  | 0.85                     | 0.17                      |
|                             | Pre 2010 HAI-GMT | < median | 22.8 (16.7;31.2)         |
|                             | ≥ median | 28                      | 148.6 (109.4;201.8)       |
|                             | p-value  | <0.001                   | < 0.001                   |

Continuous variable were tested as categorical variable based on biological cut-off or on medians.
HAI: hemagglutination inhibition; 95%CI: 95% Confidence Interval; Pre 2010: before 2010/2011 immunization; Post 2010: 4 weeks after 2010/2011 immunization; PI: protease inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; ART: antiretroviral therapy; HAART: highly active antiretroviral therapy; MMF: mycophenolate mofetil; EC-MPA: enteric-coated mycophenolic acid.

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Table 4. Multivariate regression analyses of anti-influenza A/09/H1N1 HAI titre determinants.

| A. Healthy subjects Multivariate analyses | Pre 2010 HAI-GMTs | Estimate (se) | Relative change | p |
|------------------------------------------|------------------|---------------|-----------------|---|
| Age (per 10 years)                       | -0.28 (0.08)     | -25%          | <0.001          |   |
| Post 2010 HAI-GMTs                       |                  |               |                 |   |
| Age (per 10 years)                       | -0.23 (0.08)     | -20%          | 0.005           |   |
| CDC B                                    | 0.29 (0.20)      | 34%           | 0.15            |   |
| CDC C                                    | 0.07 (0.25)      | 7%            | 0.77            |   |
| ART (compared to no ART)                 | 0.87 (0.34)      | 139%          | 0.01            |   |
| CDC A                                    |                  |               |                 |   |
| CDC B                                    | 0.18 (0.22)      | 19%           | 0.43            |   |
| CDC C                                    | 0.40 (0.27)      | 49%           | 0.14            |   |
| ART (compared to no ART)                 | 0.92 (0.37)      | 151%          | 0.01            |   |
| CDC B                                    |                  |               |                 |   |
| CDC C                                    |                  |               |                 |   |
| ART (compared to no ART)                 |                  |               |                 |   |

| B. HIV                                   | Pre 2010 HAI-GMTs | Estimate (se) | Relative change | p |
|------------------------------------------|------------------|---------------|-----------------|---|
| Age (per 10 years)                       | -0.06 (0.08)     | -5%           | 0.50            |   |
| CD4 > 350                                | 0.63 (0.24)      | 88%           | 0.008           |   |
| Nadir CD4 > 100                          | 0.03 (0.23)      | 3%            | 0.91            |   |
| CDC A                                    |                  |               |                 |   |
| CDC B                                    | 0.29 (0.20)      | 34%           | 0.15            |   |
| CDC C                                    | 0.07 (0.25)      | 7%            | 0.77            |   |
| ART (compared to no ART)                 | 0.87 (0.34)      | 139%          | 0.01            |   |

C. Kidney recipients

| Pre 2010 HAI-GMTs | Estimate (se) | Relative change | p |
|-------------------|---------------|-----------------|---|
| Age (per 10 years)| -0.24 (0.09)  | -22%            | 0.006          |   |
| CD4 > 350         | 0.25 (0.26)   | 29%             | 0.34           |   |
| Nadir CD4 > 100   | -0.23 (0.25)  | -21%            | 0.36           |   |
| CDC A             | 0.29 (0.22)   | 19%             | 0.43           |   |
| CDC C             | 0.40 (0.27)   | 49%             | 0.14           |   |
| ART (compared to no ART) | 0.92 (0.37) | 151% | 0.01 |

Table 4. Multivariate regression analyses of anti-influenza A/09/H1N1 HAI titre determinants.

compensation, an exclusion recruitment criterion. As non-adjuvanted influenza vaccines essentially reactivate pre-existing memory B cells, it suggests that in HCs 1 dose of Pandemrix® drove more B cells towards antibody-secreting plasma cells than memory B cells.

It is thus quite remarkable that HIV and SOT patients responded with higher titers to the non-adjuvanted 2010/2011 seasonal vaccine as to Pandemrix® in 2009/2010. This is unlikely to reflect higher immune competence: the CD4 counts of HIV patients were not higher in 2010/2011 and the immunosuppression of SOT patients was essentially unchanged. Furthermore, immune competence would not be higher in HIV/SOT patients than in controls, in whom 2010/2011 responses remained lower.

As primary 2010/2011 responses to influenza A/09/H3N2 were lower and not different among patients and controls, our observations indicate a true enhancement of responses to the A/09/H1N1 strain. Why did patients raise better memory responses than healthy subjects? One key difference is that patients were primed in 2009 with 2 doses and HC with 1 dose of Pandemrix®. This second primary dose implies an additional exposure to A/09/H1N1 and to the AS03 adjuvant. Whether 2 doses of non-adjuvanted H1N1 vaccine would increase subsequent memory responses may not be excluded. But memory B-cells were indeed reported as more numerous in HIV patients immunized with Pandemrix® than non-adjuvanted A/09/H1N1 vaccines [31]. Our field study was not planned to compare the influence of one versus two doses of Pandemrix® on memory responses, which would not have been allowed by the Swiss authorities. Nevertheless, the most likely explanation to our observations is that 2 doses of AS03-adjuvanted vaccines provided a more efficient induction of memory responses than a single vaccine dose. This was sufficient to overcome the potential deficiencies of the B cell compartment caused by HIV infection. It even proved beneficial in SOT patients, although their antibodies persisted/were reactivated at lower levels than in HIV patients similarly primed with 2 doses of adjuvanted pandemic vaccine, indicating lower immune competence. That adjuvanted vaccines may improve antibody persistence and memory responses of immunocompromised patients is worth studying further.

Supporting Information

Figure S1 Study Flow Chart.

Table S1 Description of Adverse Reactions.

Protocol S1 Trial Protocol.

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Memory Responses to Pandemic Influenza Vaccine

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Conceived and designed the experiments: CAS CVD LB BH AC. Performed the experiments: MB SM KH PMS SY. Analyzed the data: CC CD. Contributed reagents/materials/analysis tools: MB SY LK CAS. Wrote the paper: CAS. Reviewed the manuscript and agreed to its submission: CAS CVD CC CD MC OC SM KH PMS SY LB BH AC. Contributed to data acquisition: MB MC OC PG SM KH PHS SY.

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