Cellular and Humoral Immune Responses to Pandemic Influenza Vaccine in Healthy and in Highly Active Antiretroviral Therapy-Treated HIV Patients

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Abstract

Influenza vaccination is recommended for HAART-treated HIV patients to prevent influenza illness and complications. Due to the known ability of T cells to mediate a broadly cross-reactive response, vaccination effectiveness in cell-mediated immune (CMI) response induction is a main objective in new influenza vaccination strategies. Nevertheless, data on CMI responses after pandemic vaccination in HIV subjects are still missing. In the present study, the ability of a single dose of adjuvanted pandemic influenza vaccine to induce humoral and CMI responses was compared in HAART-treated HIV patients and in healthcare workers. Healthcare workers (HCW, $n = 65$) and HAART-treated HIV patients (HIV, $n = 67$) receiving pandemic vaccination were enrolled and analyzed before (t0) and after (t1) vaccination. The analysis of strain-specific humoral response was performed by HAI assay; CMI against pandemic (A/H1N1/Cal/09) and seasonal (A/H1N1/Brisb/07 and A/H3N2/Brisb/07) strains was analyzed by ELISpot and intracellular staining followed by flow cytometry. Pandemic vaccination was effective in inducing both humoral and cell-mediated responses in HAART-treated HIV patients as well as in HCWs. A large fraction of both HCWs and HIV-infected patients showed a T cell response to the pandemic strain before vaccination, suggesting possible previous exposure to A/H1N1/pdm/09 and/or cross-reactive T cells. Notably, pandemic vaccine was also able to boost cross-reactive immune responses to seasonal strains. Finally, a weaker boost of both strain-specific and cross-reactive T cell immunity was found in individuals showing a higher baseline response. These data show the effectiveness of adjuvanted pandemic vaccine to induce both humoral and cellular (strain-specific and cross-reactive) immune responses in HIV patients similar to HCWs.

Introduction

THREE GLOBAL INFLUENZA PANDEMICS occurred in the twentieth century caused by different influenza A strains: H1N1 in 1918, H2N2 in 1957, and H3N2 in 1968.^{1,2} More recently, in early April 2009, a new influenza A virus (A/H1N1/pdm/09) emerged in California and Mexico,³ quickly spreading worldwide through human-to-human transmission, and generating the first influenza pandemic of the twenty-first century.^{4,5} Clinical presentation of 2009 H1N1 influenza was generally mild as the majority of infected persons worldwide experienced uncomplicated influenza-like illness with a full recovery in 1 week, even

without medical treatment.⁶ Nevertheless, several risk factors for severe clinical presentation have been identified, such as young age, diabetes, pregnancy, and immune system deficiency.⁷ In particular, advanced HIV infection, such as acquired immune deficiency (AIDS), is associated with greater seasonal influenza-related morbidity and mortality, but the risks in well-controlled HIV patients are largely unknown.^{8–10} The clinical presentation of the 2009 H1N1 infection in HIV-infected individuals showed similar outcomes in well-controlled highly active antiretroviral therapy (HAART)-treated HIV patients and in healthy donors.^{11,12} In contrast, patients with advanced HIV disease experienced severe 2009 H1N1 infection and increased mortality.¹³

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TABLE 1. BASELINE CLINICAL CHARACTERISTICS OF ENROLLED PATIENTS

	Gender (M/F)	Age (years)	2009 seasonal flu vaccination	CD4 (cells/ μ l)	HIV-VL (copies/ml)
HCWs <i>n</i> = 65	33/32	45 (27–65)	17/65 (26.1%)	n.t.	n.t.
HIV <i>n</i> = 67	58/9	47 (28–71)	36/67 (53.7%) ^a	463 (218–2.081)	Undetected: 42 pts Detected below 40 cp/ml: 10 pts 40–10 ³ cp/ml: 5 pts > 10 ³ cp/ml: 8 pts

Sixty-five healthcare workers (HCWs) and 67 HIV-infected patients (HIV) were enrolled in this study. All HIV patients were undergoing HAART.

^aHIV vs. HCWs, Chi-square test, $p < 0.005$.

n.t., not tested; pts, patients.

Influenza vaccination is recommended for HIV-infected persons,¹⁴ but its effectiveness depends on host factors such as CD4 T cell counts and HIV viral load.¹⁵ Studies assessing the efficacy of seasonal influenza vaccination showed good effectiveness both in HIV-1-infected persons¹⁶ as well as in healthy adults.¹⁷ In pandemic A/H1N1 influenza vaccine studies, the data are contrasting. In fact, several studies on the efficacy of MF59-adjuvanted 2009 pandemic A/H1N1 influenza vaccine (A/H1N1/Cal/09) suggested a lower rate of influenza seroconversion in HIV-infected individuals (36.4–69%) as compared with healthy donors (>79%),^{18–20} even in patients with high CD4 T cell counts.²¹ In contrast, other studies reported a good antibody response in successfully HAART-treated HIV patients.^{22,23} However, all these studies addressed the humoral response only, and no information about the ability of pandemic vaccine to elicit a strain-specific and cross-reactive T cell response is available in HIV-infected individuals.

The induction of effective T cell immunity by natural influenza infection as well as by vaccination represents a key step for reducing the severity of symptoms and preventing complications, such as secondary bacterial infections.^{23–29} Indeed, a profound impairment of T cell response was found to be associated with a severe or fatal clinical course of pandemic 2009 H1N1 infection in healthy individuals, suggesting a strict link between T cell competence and clinical severity of pandemic influenza infection.³⁰ Moreover, it was shown that T cell immunity plays a pivotal role in the context of cross-immunity for its ability to recognize highly conserved internal epitopes,³¹ thus contributing to cross-immunity to different influenza strains.³² Indeed, a vaccine providing protective immunity to highly conserved internal antigens could provide longer-lasting protection against multiple influenza subtypes.³³

The aim of the present study was to evaluate the effectiveness of the pandemic vaccine to induce both strain-specific and cross-reactive T cell immunity in healthy donors and in HAART-treated HIV patients. In particular, the frequency of T cells specific for the pandemic strain (A/California/7/2009/H1N1) or for H1N1 and H3N2 seasonal strains (A/Brisbane/59/2007/H1N1, A/Brisbane/716/2007/H3N2) before and 1 month after pandemic vaccination was analyzed in healthy donors and in HAART-treated HIV patients, in the background of classical humoral response.

Materials and Methods

Study population

Healthcare workers (HCWs, *n* = 65) and HAART-treated HIV patients (HIV, *n* = 67) receiving MF59-adjuvanted 2009 pandemic A/H1N1 influenza vaccine (Novartis, Siena, Italy) at the Spallanzani Institute were enrolled. The study was approved by the local Ethical Committee and all participants gave written informed consent. Baseline characteristics of healthcare workers (HCWs) and of HIV populations are reported in Table 1. All enrolled HCWs were negative for HIV infection. Inclusion criteria for HIV-infected patients were CD4 > 200 cells/ μ l and HAART. As described in Table 1, HIV viral load was undetected in the majority of patients (*n* = 41), was detected below quantification threshold (< 40 cp/ml) in 10 patients, and was quantifiable in 15 patients. Blood samples were obtained before (t0) and 1 month after vaccination (t1). Interestingly, a significantly higher proportion of HIV patients underwent 2009 seasonal vaccination in comparison to HCWs (Table 1, $p < 0.005$). The vaccine formulation was Focetria (Novartis, Siena, Italy) containing inactivated, adjuvanted A/California/7/2009 (H1N1) strain. Each 0.5-ml vaccine dose contains 15 μ g HA of H1N1 in phosphate-buffered saline and excipients. A single dose of the vaccine was administered intramuscularly.

Viral strains and antigens

The subunit preparations of the influenza antigens were kindly provided by Novartis, Siena, Italy. All the preparations consisted of highly purified HA and NA from egg-grown viruses. For the hemagglutination inhibition (HAI) test, an antigen preparation from A/H1N1/California/7/2009 was used. In the influenza-specific T cell immunity study, antigen preparations from the pandemic (A/H1N1/California/7/2009) and from seasonal strains (A/H1N1/Brisbane/59/2007 and A/H3N2/Brisbane/7/6/2007) were used. For simplicity, from this point onward, the viral strains will be abbreviated as H1N1/Cal/09, H1N1/Brisb/07, and H3N2/Brisb/07, respectively.

Hemagglutination inhibition assay

All serum samples were tested for detection of anti-A/H1N1/pdm/09 antibodies by HAI assay. HAI assays were performed in V-bottom 96-well plates using turkey

TABLE 2. BASELINE CLINICAL CHARACTERISTICS OF HEALTH CARE WORKERS AND HIV SUBGROUPS USED FOR CELL-MEDIATED IMMUNE ANALYSIS

	Gender (M/F)	Age (years)	2009 seasonal flu vaccination	CD4 (cells/ μ l)	HIV-VL
HCWs	12/11	46 (33–65)	2/23 (9%)	n.t.	n.t.
Subgroup for CMI study <i>n</i> = 23					
HIV	17/2	46 (36–71)	11/19 (58%) ^a	550 (265–2081)	Undetected: 11 pts Detected below 40 cp/ml: 5 pts 40–10 ³ cp/ml: 2 pts >10 ³ cp/ml: 1 pt
Subgroup for CMI study <i>n</i> = 19					

Twenty-three healthcare workers (HCWs) and 19 HIV-infected patients (HIV) were enrolled in this study. All HIV patients were undergoing HAART.

^aHIV vs. HCWs, Chi-square test, $p < 0.005$.

CMI, cell-mediated immune; n.t., not tested; pts, patients.

erythrocytes, as described.^{34,35} Positive control serum was included in all assays and was anti-A/California/7/2009 (H1N1) HA serum (SHEEP 506) 09/142 prepared at the National Institute for Biological Standards and Control (UK) and kindly provided by Novartis Vaccines.

All specimens were tested in serial 2-fold dilutions (from 1:10 up to 1:320). The serological assay was considered positive when the antibody titer exceeded the minimum dilution (1:10); the cut-off value for protection was set at 1:40 according to EMEA criteria.³⁶ For computational purpose, a value of 1:5 was assigned to the samples that were HAI negative at 1:10. The reciprocal of the dilutions of the HAI was transformed to log₂ for statistical evaluation.

Prevaccination and postvaccination serum samples from each individual were tested at the same time.

Cell-mediated immunity

The cell-mediated immune response was tested on a subgroup of HCWs and HIV-infected patients as described in Table 2. No significant differences in HIV viral load or CD4 T cell number were found between the subset of HIV patients tested for cell-mediated immunity (CMI) and the whole group of HAART-treated HIV patients.

The CMI response was assessed by detecting interferon-gamma (IFN- γ) production using an enzyme-linked immunosorbent spot-forming cell assay (ELISpot). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Ficoll- Isopaque, Pharmacia Biotech, Piscataway, NJ) and frozen in liquid nitrogen according to standard procedures. PBMCs were thawed in culture medium (RPMI 1640, 10% FCS, 2 mmol/liter L-glutamine) and assessed for vitality by Trypan Blue exclusion, counted, and plated at 3×10^5 cells/well in ELISpot plates (AID GmbH, Strabberg, Germany). Then, PBMCs were stimulated for 20–24 h with 1 μ g/ml of influenza virus antigens (H1N1/Cal/09, H3N2/Brisb/07, and H1N1/Brisb/07) in the presence of 1 μ g/ml α CD28 and α CD49d (IgG1, clones CD28.2 and 9f10, respectively; Becton Dickinson, Mountain View, CA). At the end of the incubation, the ELISpot assay was developed according to manufacturer's instructions. Spontaneous cytokine production (background) was assessed by incubating cells with α CD28 and α CD49d. Results are expressed as spot forming cells (SFC)/10⁶ PBMCs in stimulated cultures after subtracting spontaneous background.

Forty SFC, i.e., average background response +2 SD, was arbitrarily established as the cut-off SFC value for a positive T cell response. Although retesting samples on separate occasions gave reproducible results, t0 and t1 samples for each participant were tested simultaneously to further reduce test variability.

In selected HCWs ($n=3$) and HIV patients ($n=3$), the phenotypic characterization of flu-specific T cells was performed by intracellular staining and flow cytometry. Briefly, thawed PBMCs were stimulated for 18 h with the same influenza antigens used in the ELISpot assay in the presence of 1 μ g/ml α CD28 and α CD49d and of Brefeldin-A (10 μ g/ml, Sigma, St. Louis, MO). At the end of incubation, PBMCs were labeled with monoclonal antibodies (mAbs) specific for CD4 (APC-Cy7, clone RPA-TA) and CD8 (PercP, clone HIT8A), and after fixation/permeabilization, stained with mAb against IFN- γ (APC, clone B27) and acquired on a FACS-Canto II cytometer (BD Biosciences).

Statistical analysis

Statistical analysis was performed using nonparametric tests: the Mann-Whitney test when comparing HCWs vs. HIV and the Wilcoxon test when comparing t0 vs. t1 within the same group. Differences were considered significant when the p -value was less than 0.05. Statistical evaluations were performed by GraphPad Prism software (GraphPad).

Results

Humoral immunity in HCWs and HAART-treated HIV patients before and after pandemic H1N1 vaccination

HAI activity was analyzed in 65 HCWs and 67 HAART-treated HIV patients before (t0) and 1 month (t1) after specific pandemic vaccination. In Table 3, the frequency of subjects showing protective titer ($\geq 1:40$) at t0 and t1 is shown. Before vaccination, a higher percentage of HIV patients presenting a protective HAI titer was observed when compared with HCWs (HIV: 71.6% vs. HCWs: 47.7%, $p=0.005$). These observations may be explained by considering either possible exposure to A/H1N1/pdm/09 virus during the early pandemic phase and/or cross-reactive antibodies induced by seasonal influenza vaccination. Indeed, a higher frequency of enrolled HIV persons underwent seasonal vaccination in the previous year when compared with HCWs (53.7% vs. 26.1%,

TABLE 3. HUMORAL RESPONSE AGAINST H1N1/Cal/09 BEFORE AND AFTER PANDEMIC VACCINATION

	HAI $\geq 1:40$ (%)		GMT	
	t0	t1	t0	t1
HCWs	47.7%	98.4%	25.4	106.9*
HIV	71.6% [†]	100%	32.3	109.9*

The frequency of patients presenting an HAI titer $\geq 1:40$ before (t0) and after pandemic vaccination (t1) and geometric mean titer (GMT) are shown.

* $p < 0.0001$ vs. t0.

[†] $p = 0.005$ vs. HCW.

respectively, $p < 0.005$). After vaccination (t1), almost all subjects (either HCWs or HIV-infected) reached the protective antibody titer. Moreover, the geometric mean titer (GMT) significantly increased after vaccination in both HCWs and HIV patients (Table 3). However, the percentage of subjects showing a ≥ 4 -fold increase of HAI titer was higher in HCWs as compared to HIV-infected patients (83.1% vs. 58.2%, $p = 0.0073$), probably resulting from a higher baseline HAI titer in the latter group. No correlation between age and antibody titers was found in HCWs and in HIV patients (data not shown).

Baseline influenza specific T cell response in HCWs and HAART-treated HIV patients

A baseline analysis of CMI against the pandemic (H1N1/Cal/09) and two seasonal (H1N1/Brisb/07 and H3N2/Brisb/07) strains was performed in 23 HCWs and 19 HAART-treated HIV patients by ELISpot assay. No significant differences in HIV viral load or CD4 T cell number were found between the subset of HIV patients tested for CMI and the whole group of HIV patients (Tables 1 and 2).

At baseline, a large proportion of both HCWs and HIV showed a significant T cell-specific response (> 40 SFCs/ 10^6 PBMCs) against pandemic H1N1/Cal/09 (52.1% and 63.1%, respectively), probably as a result of either exposure to A/H1N1/pdm/09 virus during the early pandemic phase and/or to the activation of T cells cross-reactive to seasonal influenza antigens.³⁷ Moreover, as expected, both HCWs and HIV showed a T cell response specific for seasonal H1N1/Brisb/07 (65.2% and 89.5%, respectively) as well as for H3N2/Brisb/07 (60.9% and 68.4%, respectively), probably due to past infections as well as to seasonal vaccination history. On the whole, no significant differences between the percentage of responding HCWs and HIV patients were found in baseline T cell response to pandemic and seasonal strains.

Moreover, we analyzed the extent of baseline individual response to different influenza strains (Fig. 1). ELISpot images of a representative experiment are shown (Fig. 1A). A higher frequency of H1N1/Brisb/07-specific T cells was observed in HIV patients as compared to HCWs [HIV: median 81.5 (IQR: 40–303) vs. HCWs: 43 (IQR: 11.5–121.5), $p < 0.01$, Fig. 1B]. We wondered whether this difference could be explained by the higher percentage of HIV patients receiving the last seasonal influenza vaccination (in CMI-analyzed subgroups: HIV: 11/19, 58% vs. HCWs: 2/23, 9%, $p < 0.0006$); no difference in H1N1/Brisb/07-specific T cell response was observed by

FIG. 1. Baseline T cell response to different influenza strains in healthcare workers (HCWs) and HIV-infected subjects (A). The number of H1N1/Cal/09-specific, H1N1/Brisb/07-specific, and H3N2/Brisb/07-specific T cells was evaluated before vaccination by ELISpot assay in HCWs ($n = 23$, white bars) and in HIV-infected subjects ($n = 19$, gray bars) (B). Results are indicated as SFC/ 10^6 peripheral blood mononuclear cells (PBMCs). Statistical analysis was performed using the nonparametric Mann–Whitney assay. * $p < 0.05$.

comparing 2009 vaccinated vs. unvaccinated patients (data not shown), suggesting other possible causes such as a higher natural infection rate and/or higher susceptibility in the HIV patient group. In contrast, the CMI response to H1N1/Cal/09 and H3N2/Brisb/07 was similar in HCWs and in HIV-infected patients.

Pandemic vaccination boosted vaccine-specific and cross-reactive T cell immunity

We explored pandemic vaccination efficiency in boosting vaccine-specific (Fig. 2) and cross-reactive (Figs. 3 and 4) T cell-mediated immunity in HCWs and HIV by comparing T cell responses at t0 and at t1 by ELISpot assay. ELISpot images of a representative experiment are shown (Fig. 2A). After vaccination (t1), a 2-fold increase in the frequency of antigen-specific T cells over baseline (t0) was arbitrarily considered significant³² and is indicated by an asterisk in the figures. According to this threshold, an increased frequency of IFN- γ -producing T cells specific for H1N1/Cal/09 was observed after vaccination in 12/23 (52.2%) HCWs (Fig. 2B) and in 13/19 (68.4%) HIV patients (Fig. 2C). Accordingly, the number of H1N1/Cal/09-specific T cells significantly increased after vaccination in both HCWs [Fig. 2, inset in A, t1: median 110 (IQR: 13–283) vs. t0: median 27 (IQR: 5–53), $p < 0.003$] and HIV patients [Fig. 2, inset in B, t1: median 113 (IQR: 50–273) vs. t0: median 33 (IQR: 13–97), $p < 0.002$], suggesting a comparable

FIG. 2. Pandemic H1N1 vaccine induced strain-specific T cell response in HCWs and HIV-infected subjects (**A**). The number of H1N1/Cal/09-specific T cells was analyzed by ELISpot assay before (t0, white bars) and after vaccination (t1, gray bars). Specifically, the individual responses of 23 HCWs (**B**) and of 19 HIV-infected subjects (**C**) are shown. Moreover, Wilcoxon paired (t1 vs. t0) statistical comparisons in HCWs and HIV are shown in the insets.

capability of HCWs and HIV in expanding strain-specific T cells. Similar results were obtained by normalizing SFCs to individual CD4 T cell counts (data not shown). Interestingly, the extent of CMI response after vaccination reached similar strength in both groups [HIV: median 113 (IQR: 50–273) vs. HCWs: 110 (IQR: 13–283)].

Pandemic vaccination was also able to induce cross-reactive T cells against seasonal H1N1/Brisb/07 in 15/23 (65.2%) HCWs (Fig. 3B) and in 10/19 (52.6%) HIV patients (Fig. 3C). ELISpot images of a representative experiment are shown (Fig. 3A). However, when evaluating individual responses, pandemic vaccination boosted H1N1/Brisb/07-specific T cell response in HCWs (Fig. 3, inset in B) but not in HIV-infected persons (Fig. 3, inset in C), probably due to the higher baseline response observed in the HIV population. Nevertheless, the extent of CMI response after vaccination was similar in both groups [HIV: median 160 (IQR: 38–320) vs. HCWs: 160 (IQR:

27–357)]. Notably, five HIV patients (#3, #6, #7, #15, and #19) showed T cell response against H1N1/Brisb/07 before vaccination, which decreased after vaccination: this was not associated with any clinical parameter such as CD4 T cell count or viral load (data not shown).

Moreover, we analyzed the ability of pandemic vaccine to improve cell-mediated cross-immunity against H3N2/Brisb/07. ELISpot images of a representative experiment are shown (Fig. 4A). A smaller number of both HCWs (7/23, 30.4%, Fig. 4B) and HIV (8/19, 42%, Fig. 4C) showed a 2-fold increase of H3N2/Brisb/07-specific T cell response. Nevertheless, a slight improvement of this response was observed after pandemic vaccination in both HCWs and HIV patients (Fig. 4, insets in B and C). Interestingly, the extent of the CMI response after vaccination was similar in both groups [HIV: median 83 (IQR: 10–270) vs. HCWs: 60 (IQR: 23–160)].

FIG. 3. Pandemic H1N1 vaccine induced cross-reactive T cell response in HCWs but not in HIV patients (**A**). The number of H1N1/Brisb/07-specific T cells was analyzed by ELISPOT assay before (t0, white bars) and after vaccination (t1, gray bars). Specifically, the individual responses of 23 HCWs (**B**) and of 19 HIV-infected subjects (**C**) are shown. Moreover, Wilcoxon paired (t1 vs. t0) statistical comparisons in HCWs and HIV are shown in the insets.

Finally, a possible correlation among individual T cell responsiveness against pandemic and seasonal strains after vaccination (t1) was analyzed. As shown in Fig. 5, CMI responses against seasonal strains were highly correlated (Fig. 5A, $p < 0.0001$). Notably, the T cell response directed against pandemic H1N1/Cal/09 was correlated with the T cell response against both seasonal strains (H1N1/Brisb/07, Fig. 5B, $p < 0.0001$; H3N2/Brisb/07, Fig. 5C, $p < 0.0001$), confirming the ability of pandemic vaccination to boost cross-reactive T cell responses against other influenza strains.

No significant correlation was observed between the *in vitro* response to influenza strains and CD4 levels or HIV-RNA viral loads in HIV-infected patients. In addition, no correlation between influenza-specific CD4 T cells and humoral responses was observed in these patients. Finally, no correlation between age and CMI response was found in HCWs and in HIV patients (data not shown).

T cell response to vaccine may depend on individual baseline response

We wondered whether baseline responsiveness could affect vaccine take. To determine this, T cell response to vaccine, evaluated by using an efficacy index (SFCs at t1/SFCs at t0), was compared in HCWs and HIV patients divided on the basis of their baseline response (lower than 60 SFCs: low responder, LR; higher than 60 SFCs: high responder, HR). As shown in Fig. 6B, in HIV patients, a lower response at baseline was associated with better vaccine responsiveness both in terms of strain-specific H1N1/Cal/09-specific T cells [LR: median 4.03 (IQR: 2.22–18.70) vs. HR: 1.15 (IQR: 0.44–1.80), $p < 0.01$] and cross-reactive H1N1/Brisb/07 T cells [LR: median 3.40 (IQR: 1.76–5.40) vs. HR: 0.79 (IQR: 0.15–2.19), $p < 0.02$] and H3N2/Brisb/07 T cells [LR: median 2.40 (IQR: 1.20–5.10) vs. HR: 1.18 (IQR: 0.43–1.76), $p = 0.06$]. Although not significant, a similar trend was also obtained in HCWs (Fig. 6A).

FIG. 4. Pandemic H1N1 vaccine induced cross-reactive T cell response in HCWs and in HIV patients (**A**). The number of H3N2/Brisb/07-specific T cells was analyzed by ELISpot assay before (t0, white bars) and after vaccination (t1, gray bars). Specifically, the individual responses of 23 HCWs (**B**) and of 19 HIV-infected subjects (**C**) are shown. Moreover, Wilcoxon paired (t1 vs. t0) statistical comparisons in HCWs and HIV are shown in the insets.

Discussion

T cell immunity plays a central role in fighting influenza infection because of both its ability to aid antibody production and to provide cross-strain protection against different influenza A viruses.^{33,38–40} A defective T cell immunity has been shown to be associated with more severe or a fatal course of influenza-related disease.³⁰ In this context, the ability of influenza vaccination to boost T cells specific for different influenza strains in HIV-infected persons could represent useful information to evaluate vaccine effectiveness in immunocompromised hosts.

In the present study, the ability of a single dose of adjuvanted pandemic influenza vaccine to induce humoral and cellular immune responses was compared in HAART-treated HIV patients and in HCWs. Serological analysis showed good vaccine effectiveness in inducing humoral response in HIV-infected individuals, as 100% of them reached protective titer

after one dose of pandemic vaccination. Similar results were shown in other studies, as a single shot of pandemic vaccination in successfully HAART-treated HIV-infected adults generated an antibody response within 21 days, with rates comparable to healthy adults.^{22,23} In contrast, other work has suggested a lower response in HIV-infected individuals, even in successfully treated patients.^{18–20} It may be speculated that the apparent discrepancy is mainly attributable to the baseline level of reactivity to influenza virus antigen that is clearly higher in the HIV group.

Although vaccine effectiveness is currently evaluated by measuring its ability to elicit a protective antibody response, T cell response analysis may be critical in evaluating the ability of vaccine to elicit a broader, cross-reacting response. Thus, the main result of our work is that HAART-treated HIV patients were able to effectively respond to pandemic vaccination by expanding H1N1/CA-specific T cells, similar to healthy individuals. Notably, before vaccination, H1N1/CA-specific

FIG. 5. Correlation between T cell responses to pandemic and seasonal strains. CMI postvaccination (t1) responses against the different influenza strains [(A) H1N1/Brisb/07, (B) H1N1/Cal/09, and (C) H3N2/Brisb/07] were correlated. Pearson's r^2 and significance are shown, as well as the calculated regression line.

T cells were found in a large percentage of both HCWs and HIV-infected patients, probably due to very recent exposure to the pandemic virus and/or to cross-reactive T cells induced by previous infection or seasonal vaccination.^{32,41} Moreover, pandemic vaccination was able to significantly boost these cells in a high percentage of both HIV-infected patients and HCWs, thus showing effectiveness in inducing specific cellular immunity. Interestingly, the response rate induced by pandemic vaccine in HCWs was even higher than the response previously shown after seasonal vaccination, sug-

gesting a greater effectiveness of pandemic in comparison to seasonal vaccine.³²

Cellular immune responses play a central role in fighting influenza infection, which is considered a critical target to improve vaccination effectiveness and to induce cross-strain immunity.⁴² It is known that influenza-specific T cells are unable to prevent reinfection per se, but can mediate faster viral clearance and provide a substantial degree of protection.^{38,39,43–45} Indeed, during an A/H1N1/pdm/09 pandemic, a severe or fatal course of influenza infection was associated with a complete loss of T cell response capability.³⁰ Moreover, the cellular immune response was able to mediate cross-immunity by recognizing viral peptides highly conserved among different influenza strains. In animal models, a protective role of cross-reactive T cells in recognizing and protecting against different lethal influenza infections^{46–48} including pandemic H1N1 infection^{40,49} is well demonstrated. Cellular cross-immunity was also found in humans^{32,41,50,51} and can be boosted by seasonal vaccination.^{32,41,52} Recently, functional A/H1N1/pds/09-specific CD4 T cells were shown in donors not exposed to the pandemic virus, probably due to the high number of conserved epitopes between pandemic and seasonal H1N1 vaccine strains.^{37,53} Interestingly, in the present study we showed the ability of pandemic vaccination to boost cross-reactive H1N1/Brisb/07- and H3N2/Brisb/07-specific T cell responses. Moreover, we found a highly significant correlation in postvaccination CMI responses between pandemic and seasonal strains, suggesting that individual responses to pandemic vaccine also influence T cell cross-reactivity against other influenza strains probably through epitope sharing mechanisms.^{54,55} In some subjects, pandemic vaccination resulted in a higher induction of cross-reactive rather than of strain-specific T cells. Other studies are required to clarify molecular mechanisms responsible for this issue such as individual epitope presentation variability that may affect individual T cell responses. Cross-reactive T cells are able to protect animals from several influenza strains and can be induced by regular vaccination in humans, thus representing an optimal target for new improved vaccination strategies.^{54,55}

Finally, an association between baseline CMI response and the ability of vaccine to elicit T cell responses was shown in HIV patients. A similar trend was also found in HCWs, not reaching statistical significance probably due to the small sample size. Many different mechanisms may explain this result, such as hyperstimulation-induced T cell anergy, expansion/deletion of T cell clones, and trafficking to mucosal surfaces. Interestingly, several observations showed that the stimulation of preactivated T cells with high doses of antigen could induce T cell anergy or activation-induced cell death.^{32,56} A key role of T cell preactivation in driving subsequent responses was also demonstrated by the observation that repeated exposure to the same influenza strain results in limited boosting of cellular immune responses.⁵⁷ A clinical study with increased sample size is required to fully characterize CMI responses to vaccine in individuals presenting high and low baseline responses.

Overall, the main result of this work shows good effectiveness of pandemic influenza vaccination in HAART-treated HIV patients in inducing humoral responses and strain-specific and cross-reactive T cells, confirming the feasibility of influenza vaccination in HIV-infected persons.

FIG. 6. Vaccine effectiveness may depend on individual baseline response. Vaccine effectiveness was evaluated by using an efficacy index (SFCs at t1/SFCs at t0) and was compared in HCWs (**A**) and in HIV (**B**) patients divided on the basis of their baseline response. LR, low responders (baseline response lower than 60 SFCs); HR, high responders (baseline response higher than 60 SFCs). Statistical analysis was performed using the nonparametric Mann–Whitney assay.

Acknowledgments

We thank all the medical and nurse staff of the National Institute for Infectious Diseases “Lazzaro Spallanzani” for their contribution to this study. We also thank the HCWs and the HIV patients who agreed to participate in the study. This study was partly funded by grants from the Italian Ministry of Health (Fondi Ricerca Corrente and Ricerca Finalizzata) and by Fondazione Cariplo (Rif. 2011-0517). All the antigen preparations were kindly provided by Novartis.

Author Disclosure Statement

No competing financial interests exist.

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