

# Influenza Vaccination of HIV-1-Positive and HIV-1-Negative Former Intravenous Drug Users

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The immunogenicity of an anti-influenza vaccine was assessed in 409 former intravenous drug user volunteers and its effect on the levels of HIV-1 RNA, proviral DNA and on CD4+ lymphocyte counts in a subset HIV-1-positive subjects was measured. HIV-1-positive individuals (n = 72) were divided into three groups on the basis of their CD4+ lymphocyte counts, while the 337 HIV-1-negative participants were allocated into group four. Haemagglutination inhibiting (HI) responses varied from 45.8 to 70% in the HIV-1-positive subjects and were significantly higher in group four (80.7% responses to the H1N1 strain, 81.6% to the H3N2 strain, and 83% to the B strain). The percentage of subjects with HI protective antibody titres ( $\geq 1:40$ ) increased significantly after vaccination, especially in HIV-1 uninfected subjects. Immunization caused no significant changes in CD4+ counts and in neither plasma HIV-1 RNA nor proviral DNA levels. Therefore, vaccination against influenza may benefit persons infected by HIV-1. **J. Med. Virol. 65:644–648, 2001.** © 2001 Wiley-Liss, Inc.

**KEY WORDS:** influenza vaccination; immunogenicity; HIV-1 RNA; HIV-1 proviral DNA; CD4+ counts

## INTRODUCTION

Influenza is an important public health problem worldwide. It is a highly contagious, ubiquitous disease that can lead to severe complications, especially in the elderly, in debilitated or chronically ill patients, in children and in immunosuppressed subjects, including those infected with the human immunodeficiency virus (HIV). In addition to its considerable impact in terms of morbidity and public health costs, influenza may cause a series of complications requiring hospital admission and leading to an excess of mortality in the subjects at high risk [Barker, 1986; Nicholson et al., 1997; Cox and Subbarao, 1999; Simonsen et al., 2000].

Vaccination is the most effective strategy for preventing influenza in high-risk subjects in terms of cost-effect and cost-benefit ratios [Nichol et al., 1994, 1998; Gross et al., 1995; Carman et al., 2000]. At present, influenza vaccination is recommended for patients with HIV-1 infection [Nichol et al., 1998; Tasker et al., 1999; Centers for Disease Control and Prevention, 2000], even though there is evidence that immunization may increase plasma HIV-1 viral load [Ho, 1992; O'Brien et al., 1995; Straprans et al., 1995; Stanley et al., 1996; Couch, 1999].

The aim of this study was to assess the immunogenicity of the 1998/1999 anti-influenza vaccine in HIV-1-positive and -negative former intravenous drug users residing in a rehabilitation community. In addition, we evaluated the effect of vaccination on HIV-1 RNA and proviral DNA levels and on CD4+ lymphocyte count in the HIV-1-positive vaccinees.

## MATERIALS AND METHODS

### Population Studied

In November 1998, an influenza vaccine was administered to 409 volunteers (290 males, 119 females, mean age 32 years, range 18–57) all of whom were former intravenous drug users (IVDUs) residing in a rehabilitation community. No participant had contraindications for vaccination as detected by clinical examinations or medical histories. All subjects had received at least one previous influenza vaccination.

Seventy-two participants (17.6%) were seropositive for HIV-1 antibodies. They were subdivided into three groups on the basis of their baseline CD4+ lymphocyte counts, according to criteria of the revised CDC 1993 classification system for HIV-infected patients [Centers for Disease Control and Prevention, 1992]. Ten patients had fewer than 200 CD4+ cells/mm<sup>3</sup> (group 1), 38 patients had levels ranging between

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200 and 500 cells/mm<sup>3</sup> (group 2) and 24 patients had more than 500 cells/mm<sup>3</sup> (group 3). Three hundred thirty-seven subjects, all negative for HIV-1 antibodies, made up group four (Table I).

In a subgroup of 40 HIV-1-positive participants, 24 of whom (60%) were on treatment with combined anti-retroviral regimen comprising two reverse transcriptase inhibitors and one protease inhibitor (HAART), HIV-1 RNA plasma levels were quantified in blood samples drawn at time 0 and at 10, 20, and 30 days after vaccination. In 31 individuals of this subgroup (21 or 67.7% of whom in therapy), peripheral blood mononuclear cells (PBMCs) associated HIV-1 proviral DNA levels were also measured at baseline and one month after immunization.

### Vaccination

Subjects were immunized using a single dose of the 1998/1999 season influenza vaccine (Inflexal V, Bern, Switzerland), containing 15 µg haemagglutinin of each of the following viral strains: A/Sydney/5/97 (H3N2), A/Beijing/262/95 (H1N1), and B/Beijing/184/93 (B). The injections were given intramuscularly in the deltoid region.

### Detection of Haemagglutination-Inhibiting (HI) Antibodies

Serum samples collected from each vaccinee, prior to (T = 0) and one month following immunization (T = 30), were assayed for haemagglutination inhibiting (HI) antibodies by standard microtitre assays [Dowdle et al., 1979] for each of the influenza strains contained in the vaccine. Pre and post-vaccination samples pairs from each subject were assayed simultaneously. The HI antibody titre was expressed as the reciprocal of the highest dilution that inhibited agglutination. Minimum response was defined as seroconversion or as a fourfold or greater increase in antibody titre in the post-immunization samples. Antibody titres  $\geq 1:40$  were considered protective against influenza infection.

To allow the calculation of the HI geometric mean titres (GMTs), a titre of 1:5 was assigned arbitrarily to non-responder vaccinees.

### CD4+ Lymphocyte Counts

CD4+ lymphocytes were counted by flow cytometry on pre- and post-immunization samples collected from

HIV-1 positive individuals (groups 1, 2, 3), according to standard methods.

### Plasma HIV-1 RNA

HIV-1 RNA was measured using a commercially available quantitative assay (Nuclisens NASBA Diagnostic, Organon Teknika, The Netherlands) according to the manufacturer's instructions. Plasma samples collected prior to and at 10, 20, and 30 days after immunization from 40 HIV-1 positive vaccinees were stored at  $-80^{\circ}\text{C}$ . Testing was performed in batches with all samples from a single subject run simultaneously. The lower limit of the assay quantification was 80 copies per ml of plasma.

### HIV-1 Proviral DNA

Proviral DNA was evaluated using an "in-house" quantitative PCR. Peripheral blood mononuclear cells were separated by Lymphoprep density gradient from 15 ml of EDTA-anticoagulated blood. DNA was extracted using a commercially available kit (Genomic DNA Isolation Kit, Sigma, Steinheim Germany). The concentration and the degree of purity of the nucleic acid obtained have been evaluated with the aid of a spectrophotometer. To verify sample competence and to determine the eventual presence of possible inhibitors, the human  $\beta$ -globin fragment (268 bp) was amplified [Bauer et al., 1991]. One microgram of proviral DNA was then amplified by two primers (SK38 and SK39) in the gag region of HIV-1 [Pieniazek et al., 1991] with reference to an external standard curve (Gene Amplifier HIV-1 Control, Perkin Elmer, Oak Brook, IL) in the range of  $10-10^4$  cp/µg genomic DNA. The detection and quantification of the amplified products were carried-out by densitometric reading (Digital Science 1D Image Analysis Software, Kodak, USA). The value of 5 cp/µg genomic DNA was assigned arbitrarily to samples presenting less than 10 cp/µg.

### Statistical Analysis

Seroconversion rates and prevalence of vaccinated individuals with HI antibody titres considered to be protective ( $\geq 1:40$ ) in the different groups of the study participants, were compared using the Chi square test ( $\chi^2$ ). Student's *t*-test was used to compare geometric mean HI antibody titres, mean CD4+ counts, mean HIV-1 RNA Log cp/ml and mean cp/µg of proviral DNA.

TABLE I. Baseline Characteristics of the 409 Former Intravenous Drug Addicts Vaccinated Against Influenza\*

Group	N°	Mean age (range)	M/F ratio	HIV seropositivity	HIV-RNA mean (log copies/ml)	CD4+ count (cell/mm <sup>3</sup> )	
						CD4 group	Mean(range)
1	10	36 (30-42)	2.3	+	3.8	< 200	141 (20-189)
2	38	37 (25-47)	3.1	+	4.3	200-500	356 (223-488)
3	24	36 (24-42)	2.4	+	4.3	> 500	693 (501-1455)
4	337	31 (18-57)	2.4	-	-	N.D.	N.D.

\*N.D., not determined.

## RESULTS

### Antibody Response to Vaccination

Pre- and post-vaccination paired-serum samples from vaccinees were tested against each of the three individual vaccine antigens. A significantly ( $P < 0.05$ ) higher percentage of HIV-1 negative vaccinees either seroconverted or developed fourfold or greater increases of HI antibodies to all three influenza strains, as compared to HIV-1 positive subjects (Table II). The frequency of HI antibody response to vaccination ranged between 45.8 and 70% among HIV-1-positive vaccinees, and was higher (80.7% response to H1N1, 81.6% to H3N2, 83% to B strains, respectively) among HIV-1 uninfected subjects. No differences were observed in rates of immune response to the vaccination among HIV-1-positive vaccinees, independent of the severity of lymphocyte depletion.

Following vaccination, a significant ( $P < 0.05$ ) increase in the percentage of subjects with protective antibody titres ( $\geq 1:40$ ) has been noted in all groups, and was more pronounced in the HIV-1-negative group. The HIV-1-negative group showed a higher response also in terms of percentages of protective antibody titres: 95.8% against the H1N1 strain, 99.1% against the H3N2 strain, and 97.6% against the B strain (Table II).

One month after vaccination, geometric mean antibody titres were significantly higher ( $P < 0.05$ ) than baseline in all three groups of HIV-1-positive individuals (mean increase between 2.6 and 6.1). The mean increase was even higher (from 7.5 to 12.1) in the group of vaccinees without HIV-1 infection (Table III).

### Patterns of CD4+ Counts

The arithmetic means of the CD4+ lymphocyte counts, in the HIV-1-positive vaccinees, measured at baseline and one month after immunization, were similar (478.4 vs 481.7 cells/mm<sup>3</sup>, respectively). Such lack of CD4+ changes during the study period has been observed in vaccinees under anti-retroviral treatment (382.8 vs. 378.9 cells/mm<sup>3</sup>,  $P > 0.05$ ) and in those untreated likewise (473.3 vs. 436.4 cells/mm<sup>3</sup>,  $P > 0.05$ ).

### HIV-1 RNA and HIV-1 Proviral DNA Levels

The mean values of plasma HIV-1 RNA did not differ during the four sampling periods (Table IV). In particular, the mean viral RNA levels remained essentially unchanged both in the groups of anti-retroviral treated and untreated subjects. Similarly, no difference has been observed in HIV-1 proviral DNA levels measured prior and after immunization in either treated, or anti-retroviral naive patients.

## CONCLUSIONS

Influenza is an important public health problem worldwide. Elderly subjects, infants, debilitated individuals, or patients with respiratory disorders, cardio-

TABLE II. Haemagglutination Inhibition (HI) Antibody Responses to Vaccination Against Influenza

Antigen	Percentage of responders (n)															
	Group 1				Group 2				Group 3				Group 4			
	T0	T30	T0	T30	T0	T30	T0	T30	T0	T30	T0	T30	T0	T30		
A H1N1	50 <sup>***</sup> (5)	68.4 <sup>***</sup> (26)	62.5 <sup>***</sup> (15)	80.7 (272)	0	50 <sup>***</sup> (5)	23.7 (9)	63.1 <sup>***</sup> (24)	25 (6)	62.5 <sup>***</sup> (15)	36.2 (122)	95.8 <sup>***</sup> (323)				
A H3N2	60 <sup>***</sup> (6)	63.1 <sup>***</sup> (24)	58.3 <sup>***</sup> (14)	81.6 (275)	50 (5)	70 <sup>**</sup> (7)	52.6 (20)	92.1 <sup>***</sup> (35)	79.2 (19)	91.6 <sup>***</sup> (22)	75 (253)	99.1 <sup>***</sup> (334)				
B	70 <sup>***</sup> (7)	50 <sup>***</sup> (19)	45.8 <sup>***</sup> (11)	83 (279)	20 (2)	80 <sup>***</sup> (8)	34.2 (13)	68.4 <sup>**</sup> (26)	29.2 (7)	62.5 <sup>**</sup> (15)	24.3 (82)	97.6 <sup>***</sup> (329)				

\*n.s.: 1 vs. 2; 1 vs. 3; 2 vs. 3.

\*\* $P < 0.05$  group 4 vs. group 1, 2, 3.

\*\*\* $P < 0.05$  in each group T0 vs. T30.

TABLE III. Geometric Means (Mean Fold Increase) of HI Antibody Titers Before (T0) and After (T30) Vaccination

Antigen	Group 1		Group 2		Group 3		Group 4	
	T0	T30	T0	T30	T0	T30	T0	T30
A H1N1	6.6	40*** (6.1)	10.4	59.7*** (5.7)	14.1	75.5*** (5.3)	18.1	219** (12.1)
A H3N2	24.6	65* (2.6)	31	157*** (5.1)	46.2	151*** (3.7)	62.8	473** (7.5)
B	12.3	74.6*** (6)	16.6	62*** (3.7)	17.3	55*** (3.2)	13.5	138** (10.2)

\**P* < 0.05 group 4 vs. group 1, 2, 3.  
 \*\**P* < 0.05 for each group T30 vs. T0.

vascular disease, or immune depression, including those infected with HIV, may develop severe complications leading to considerable morbidity and mortality [Barker, 1986; Nicholson et al., 1997; Cox and Subbarao, 1999; Simonsen et al., 2000]. Influenza vaccination is recommended for patients with HIV infection although they may exhibit lower responses and, therefore, the cost-effectiveness of such immunization for these patients remains controversial.

Conflicting evidence exists as to whether influenza vaccination can lead to increases in plasma HIV-1 RNA load and decreases CD4+ cells counts [Ho, 1992; Yerly et al., 1994; O'Brien et al., 1995; Straprans et al., 1995; Katzenstein et al., 1996; Glesby et al., 1996; Stanley et al., 1996]. Data showing increased HIV-1 RNA load and decreased CD4+ lymphocyte counts after immunization raise concern that influenza vaccination may have a potentially negative effect on the progression of HIV disease [Couch, 1999]. The observed increase in HIV-1 RNA levels could be explained by a number of mechanisms among which is the induction of replication of latent virions from cellular reservoirs. Therefore, an important aspect, insufficiently investigated to date, regards the eventual vaccine-induced stimulation of the intracellular integrated HIV-1 proviral DNA.

In our study, a trivalent influenza vaccine, issued during the 1998-1999 winter season was administered to HIV-1 positive and HIV-1 negative former drug users living in a rehabilitation community.

The percentage of HI antibody responses to the influenza vaccination ranged between 45.8 and 70% among HIV-1-positive vaccinees. Such percentages were significantly higher (from 80 to 83%) among subjects without HIV-1 infection. Similarly, the geometric mean of antibody titres and the rate of immunized individuals with protective antibody levels

(≥ 1:40) increased significantly after vaccination in all groups of vaccinees, but the increases among the HIV-1 negative individuals were more pronounced.

Past investigations have shown that the immune response of HIV-1-positive individuals to immunization varies in correlation to CD4+ counts, and that individuals with CD4+ counts < 200 cell/mm<sup>3</sup> respond poorly to anti-influenza vaccination [Kroon et al., 1994; Donovan et al., 1997; Iorio et al., 1997; Couch, 1999]. In this study, no differences in GMTs and in the percentages of vaccinees with protective levels of antibodies have been shown between HIV-1-positive vaccinees grouped according to CD4+ counts. However, the number of individuals in group 1 (CD4+ count < 200 cell/mm<sup>3</sup>, number examined = 10) in our study was too low to allow for definite conclusions. On the contrary, the immune responses to vaccination was clearly greater among HIV-1 negative vaccinees than among those infected with HIV-1 (*P* < 0.05).

Vaccination was well tolerated and no complaints of severe adverse reactions were reported.

Despite immune activation, we did not observe any evidence of immunosuppressive effect of vaccination on CD4+ lymphocytes counts, with the arithmetic means remaining essentially unchanged before and after immunization.

The mean values of plasma HIV-1 RNA viral loads did not significantly change over the month following the vaccination among the HIV-1-infected individuals examined and, in particular, such lack of change was independent from treatment with antiretroviral therapy. These results are in agreement with those reported in other published papers [Yerly et al., 1994; Glesby et al., 1996; Katzenstein et al., 1996; Donovan et al., 1997; Fowke et al., 1997; Jackson et al., 1997; Tasker et al., 1998; Fuller et al., 1999].

TABLE IV. HIV-1 RNA (Log cp/ml) and HIV-1 DNA (cp/μg DNA Genomic) in Treated and Antiretroviral Naive Vaccinees

	HIV-1 RNA (mean log cp/ml)				HIV-DNA (mean cp/μg DNA genomic)		
	T0	T10	T20	T30	T0	T30	
Untreated vaccinated (n=16)	4.53	4.43*	4.66*	4.71*	Untreated vaccinated (n=10)	35.62	8.63*
Treated vaccinated (n=24)	3.95	4.07*	3.97*	4.03*	Treated vaccinated (n=21)	25.21	26.41*
All (n=40)	4.34	4.21*	4.44*	4.5*	All (n=31)	30.41	17.52*

\**P* = n.s.

In addition, the evidence that proviral DNA levels were not significantly changed during the follow-up period seems to suggest that the antigenic stimulation represented by immunization against influenza is not sufficient to activate the replication of HIV-1. Therefore, the absence of significant modifications of both virological parameters (HIV-1 RNA and integrated proviral DNA levels) indicates that influenza vaccination does not result in any detrimental effect on the history of the underlying HIV disease.

In conclusion, even though HIV-1-positive subjects show lower immune responses to influenza vaccination in comparison to immunocompetent individuals, such immunization may be administered with benefit to people infected with the human immunodeficiency virus.

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