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Influenza surveillance in a cohort of HIV-infected children and adolescents immunized against seasonal influenza

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ABSTRACT

During the 2006–2007 season, 19 HIV-uninfected and 33 HIV-infected children and adolescents with full immunovirologic response to HAART were immunized against influenza and subsequently followed up. One month post-immunization all subjects had protective antibodies titres which persisted for the whole influenza season. Seven vaccinees (four HIV-infected and three HIV-uninfected) were found to be infected by influenza viruses during the epidemic, but disease was lab-confirmed only in two HIV-infected subjects. Both presented a benign clinical course and were infected by an A/Brisbane/10/07-H3N2-like virus. These data indicate that HIV-infected subjects benefit from routine seasonal influenza vaccination.

Current guidelines recommend that individuals infected by Human Immunodeficiency Virus (HIV) receive yearly influenza vaccination [1]. Though this recommendation is justified by increased influenza-related morbidity and mortality rates in such individuals, the clinical effectiveness of influenza vaccines in HIV-infected subjects is still poorly understood [2,3]. Since most previous studies of influenza vaccines in HIV-infected individuals focused on their immunogenicity rather than on their clinical effectiveness [2], it is crucial to set up close post-vaccination influenza surveillance to monitor the persistence of immune response and the effective immune protection against viral strains circulating during the seasonal epidemics. The aim of this study was to carry out a serological, clinical and virological surveillance of influenza in a cohort of HIV-infected children and adolescents immunized against seasonal influenza during the 2006–2007 epidemic season.

1. Materials and methods

1.1. Study design and population

This observational, prospective, open-label study considered HIV type 1-infected children and adolescents ($N = 33$, mean age: 15.5 ± 4.9 years, 17 males) and age- and gender-matched HIV-negative subjects ($N = 19$, mean age: 14.3 ± 3.1 years, 10 males) undergoing influenza vaccination in the Department of Pediatrics, University of Milan, Luigi Sacco Hospital (Milan, Italy) in November 2006. All participants were vaccinated against influenza in the previous season. HIV-infected subjects were clinically stable and had long-lasting control of viral replication and immune recovery. All showed an undetectable plasma viral load (HIV-RNA level <50 copies/ml) and had been subjected to a long duration (mean: 95 months; range: 28–117 months) highly active antiretroviral therapy (HAART).

A written informed consent form was obtained from eligible subjects and from parents or legal guardians of eligible children. All subjects were injected intramuscularly in the deltoid muscle with one dose (0.5 ml) of a trivalent virosomal adjuvanted influenza vaccine (Inflexal®V, Berna Biotech Ltd, Berne, Switzerland) for the 2006–2007 season. This vaccine contained 15 μg each of A/NewCaledonia/20/99-H1N1-like, A/Wisconsin/67/05-H3N2-like, and B/Malaysia/2506/04-like hemagglutinin.

A baseline blood sample was collected at the time of enrolment and vaccination (T0); a post-vaccination blood sample was drawn one month later (T1), and a final blood sample was obtained one month after the end of the seasonal influenza activity (T6). Post-vaccination influenza surveillance among vaccinees was conducted from November 2006 through April 2007. Participants were instructed to contact study staff in the event of illness with respiratory or systemic signs or symptoms. Oropharyngeal swabs were collected for the identification of influenza viruses.

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1.2. Definitions and outcomes

Vaccine response was defined as a fourfold or greater increase in antibodies titres between pre-immunization and post-immunization specimens (T1/T0) [4]. Antibody titres ≥1:40 were considered protective against influenza infection (seroprotection) [4].

Influenza virus infection during the epidemic period was defined as a fourfold or greater increase in antibody titres (natural booster) by comparing post-immunization and post-epidemic specimens (T6/T1) [5].

Symptomatic influenza was defined as an illness characterized by abrupt onset of fever (>38.0 °C), by one or more respiratory symptoms (non-productive cough, sore throat, rhinitis), and by one or more systemic symptoms (myalgia, headache, severe malaise). Antibiotic treatment and hospital admissions for bronchitis, pneumonia, or emphysema were considered as indicators of the illness clinical severity.

1.3. Laboratory assay

Humoral immune response was assessed by hemagglutination inhibition (HI) test using the standard microtitre assay [6]. This assay determined the antibody titres in serum against the hemagglutinin antigens for A/H1N1, A/H3N2, and B vaccine influenza strains recommended for the 2006–2007 season. The HI antibody titre was expressed as the reciprocal of the highest dilution that inhibited agglutination.

Viral RNA was purified from each oro-pharyngeal swab collected from all vaccinees with ILI (QIAmp Viral RNA kit, Qiagen GmbH, Hilden, Germany) and analyzed through a one-step real-time RT-multiplex-PCR assay [7]. The molecular characterization of A/H3 influenza viruses was performed by sequence analysis of the globular head region of the HA protein (HA1 subunit) [8]. Multiple sequence alignment was conducted using ClustalX, version 2.0. The phylogenetic tree was constructed by means of neighbor-joining method and Kimura 2-parameter model using MEGA package, version 4.0 [9].

1.4. Statistical analysis

Descriptive data were reported as percentages, HI geometric mean titres (GMTs), and 95% confidence intervals (95% CI). To allow the GMTs calculation a titre of 1:5 was arbitrarily assigned to non-responder vaccinees [4]. Strain-specific antibody titres of subjects whose serological profile revealed an influenza virus infection were excluded from GMTs calculation six months post-vaccination.

Immune responses one month post-vaccination were compared by Mid-P Exact test based on binomial distribution (seroconversion and seroprotection rates) and paired *t*-test (GMTs). A *P*-value <0.05 was considered significant (2-tailed test).

2. Results

2.1. Antibody levels after immunization

The percentages of HIV-infected vaccinees with protective HI antibodies titres ranged between 72.7% and 87.9% at baseline and reached 100% one month after vaccination. A similar figure was observed among HIV-uninfected individuals. No significant ($P > 0.05$) differences in the rates of vaccinees with protective levels of antibodies were observed between the two groups. One month after immunization, antibody GMTs against all vaccine strains were significantly higher ($P < 0.05$) than those measured at baseline in both groups (Table 1). The percentages of subjects who developed a fourfold or greater increase of HI antibodies ranged between

Table 1
Pre- and post-immunization seroprotection rates and HI geometric mean titres (GMTs) in the two groups of vaccinees. 95% confidence intervals (95% CI) are reported in brackets.

Viral strain	Group	Baseline	One month post-vaccination	Six months post-vaccination	P^a	P^b
Seroprotection rates (95% CI)						
A/H1N1[c]	HIV-infected	87.9 (73.3–96)	100 (91.3–100)	97 (85.9–99.8)	0.0376	0.3423
	HIV-uninfected	89.5 (69.4–98.2)	100 (85.4–100)	100 (85.4–100)	0.1616	0.5000
P^d		0.8955	0.6321	0.4940		
A/H3N2[e]	HIV-infected	81.8 (66–92.3)	100 (91.3–100)	97 (85.9–99.8)	0.0078	0.3423
	HIV-uninfected	89.5 (69.4–98.2)	100 (85.4–100)	100 (85.4–100)	0.1616	0.5000
P^d		0.5043	0.6321	0.4940		
B[f]	HIV-infected	72.7 (55.8–85.8)	100 (91.3–100)	100 (91.3–100)	0.0006	0.5000
	HIV-uninfected	63.2 (40.3–82.2)	100 (85.4–100)	100 (85.4–100)	0.0022	0.5000
P^d		0.4898	0.6321	0.6321		
GMTs (95% CI)						
A/H1N1[c]	HIV-infected	189.3 (56.3–322.3)	508 (310–706)	355.4 (179.7–531.2)	0.0019	0.0709
	HIV-uninfected	344.2 (103.7–584.8)	740.6 (354.7–1126.4)	533.3 (177.9–888.7)	0.0410	0.2711
P^g		0.0245	0.0759	0.0397		
A/H3N2[e]	HIV-infected	90.8 (40.3–141.2)	181.5 (97.1–265.8)	160 (67.9–252.1)	0.0164	0.3273
	HIV-uninfected	115.2 (52.5–177.9)	222.2 (71.7–372.6)	166.3 (74.1–258.5)	0.0321	0.1179
P^g		0.2062	0.1801	0.4821		
B[f]	HIV-infected	40.9 (18.9–62.8)	276.3 (139–413.5)	170.4 (83.3–257.5)	<0.0001	0.0418
	HIV-uninfected	32.1 (15.4–48.9)	247.9 (66.3–429.5)	180.8 (26–335.7)	0.0004	0.1463
P^g		0.1184	0.3963	0.4561		

Note: Data are percentages of subjects having HI antibody titre ≥1:40 or geometric means of antibody titres (GMTs) against the influenza viral strains and related 95% confidence interval.

[a] Comparison between baseline and one month post-vaccination within group (Mid-P Exact test or *t*-test based on the binomial distribution).
[b] Comparison between baseline and six months post-vaccination within group (Mid-P Exact test or *t*-test based on the binomial distribution).
[c] A/New Caledonia/20/99-H1N1-like.
[d] Comparison between HIV-infected and HIV-uninfected vaccinees (Mid-P Exact test based on the binomial distribution).
[e] A/Wisconsin/67/05-H3N2-like.
[f] B/Malaysia/2506/04-like.
[g] Comparison between HIV-infected and HIV-uninfected vaccinees (*t*-test based on the binomial distribution).