

# INFLUENZA IMMUNIZATION ELICITS SUBOPTIMAL IMMUNE RESPONSES IN HIV-INFECTED CHILDREN WITH FULLY SUPPRESSED HIV VIREMIA AND IMMUNE RECOVERY

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## ABSTRACT

**Objective:** Immune responses to vaccines are often impaired by HIV infection. HAART improved virologic and immunologic outcomes in treated children; however it is unclear whether specific immune responses to vaccine antigens are also restored. We evaluated influenza-specific humoral and cell mediated immune responses upon vaccination in HIV-infected children (HIV) on long-term successful HAART.

**Methods:** We studied 24 HIV children (median age 11.9 yr) on long-term HAART (mean exposure 85 months) and 14 age- and sex-matched healthy controls (HC). Mean CD counts and percentage were comparable between the two groups (HIV= 900 cell/ml, 36.9%; HC= 992 cell/ml, 38.8). HIV-RNA was < 50 copies/ml in all HIV children. All HIV children were unprimed and immunized with a single i.m. dose of trivalent virosome-adjuvanted influenza vaccine (Inflexal V, Berna Biotech, CH). A/H3N2, A/H1N1 and B antigen specific antibody (Ab) titres and subclasses as well as vaccine specific IFN $\gamma$  and IL-2 producing T lymphocytes (ELISPOT) were analyzed at baseline and 1(T1) and 6 (T6) months after immunization.

**Results:** Seroconversion ( $\geq 4$  fold increase in Ab titres in > 40% of subjects) and seroprotection (Ab titres  $\geq 1:40$  in >70% of subjects) were defined, 1 month after immunization, according to the European Agency for Evaluation of Medical Products criteria. Despite these criteria being fulfilled by both groups of patients, the seroconversion and seroprotection rates were constantly lower in HIV children (seroconversion= A/H3N2: 54.1 vs. 71.4; A/H1N1: 70.8 vs. 92.8; B: 70.8 vs. 85.7)(seroprotection = A/H3N2: 79.2 vs. 92.8; A/H1N1: 79.2 vs. 92.8; B: 75.0 vs. 92.8). The A/H3N2- and A/H1N1-specific Ab geometric mean titers (GMT) were significantly lower in HIV compared to HC both at T1 and T6; interestingly, a boost in vaccine-specific IgG3 (Th1-type Ab) was seen in HC but not in HIV children. Finally, vaccine specific-, IFN $\gamma$  and IL-2-producing T lymphocytes were similarly significantly reduced at both time points in HIV children compared to HC.

**Conclusions:** The virosome-adjuvanted influenza vaccine showed a comparable immunogenicity in HIV and HC. However, functional impairments of immune responses persist in HIV infection even in children with suppressed viremia and optimal immune recovery. These impairments result in the elicitation of suboptimal humoral and cellular response to immunization.

## BACKGROUND

Immune responses to vaccines are often impaired by HIV infection. HAART improved virologic and immunologic outcomes in treated children; however it is unclear whether specific immune responses to vaccine antigens are also restored.

## AIMS

The aim of this study was to compare the immunogenicity of 2005/2006 antiinfluenza vaccine in HIV-infected children on long-term successful HAART and age- and CD4+ cells count- matched healthy controls.

## STUDY POPULATION

- 24 HIV-infected and 14 healthy (HC) influenza vaccine naive children were enrolled in the study
- all children received a single dose of 2005/06 season influenza vaccine (Inflexal @ V, Berna Biotech Ltd, Berne, Switzerland)
- The study was approved by the Ethic Committee of L. Sacco Hospital of Milan, Italy

## THE EMEA CRITERIA FOR VACCINE IMMUNOGENICITY (IN ADULTS)

- Seroconversion:  $\geq 4$  fold increase in HI antibody titer with a titer of  $\geq 1:40$  being reached in >40% of the subjects
- Seroprotection: HI antibody titer of  $\geq 1:40$  in >70% of the subjects
- GMT: a >2,5-fold increase in the HI antibody GMT

## BASELINE CHARACTERISTICS OF PATIENTS AND HC

	HIV-infected children (n=24)	HC (n=14)	p value
Male / Female	9 / 15	8 / 6	0.23
Age (yrs)	12.6 (4.6)	9.7 (4.1)	0.07
CDC class A / B+C	8 / 16	NA	
CDC class 1 / 2+3	11/13	NA	
HAART exposure (months)	85 (31.98)	NA	
HIV-RNA < 50 copies/ml	24/24	NA	
CD4+ T lymphocytes (cell/ $\mu$ L)	900 (259)	992 (407)	0.40
CD4+ T lymphocytes (%)	36.9 (9.1)	38.8 (4.1)	0.45
CD8+ T lymphocytes (%)	31.5 (9.0)	24.6 (5.6)	0.01
CD19+ B lymphocytes (%)	16.1 (4.4)	19.1 (5.4)	0.07

## METHODS

- Detection of haemagglutination-inhibiting (HI) antibodies:** Serum samples were collected before, 4 and 24 weeks after immunization. Serum samples were simultaneously examined for HI antibodies using standard microtitre assays for each of the influenza strains contained in the vaccine. The HI antibody titre was expressed as the reciprocal of the highest dilution inhibiting agglutination.
- Vaccine-specific IFN $\gamma$ - and IL2-secreting CD8+ T cells (ELISPOT):** Vaccine-specific IFN $\gamma$  and IL2 release by CD8+ T cell was detected by ELISPOT stimulating PBMC with influenza vaccine in the presence of neutralizing antiCD4 monoclonal antibody. Individual IFN $\gamma$ - or IL2-producing cells were detected as dark blue or red spots using an alkaline phosphatase conjugate substrate kit, respectively. The spots were counted using an automated Elispot Reader (Aelvis). Vaccine-specific responses were reported as number of spot-forming units (SFU)/10<sup>6</sup> mononuclear cells after subtraction of background IFN $\gamma$  or IL2 secretion.
- Vaccine-specific IgG3:** Serum vaccine-specific IgG3 were detected by ELISA. Microplates were coated with influenza vaccine and peroxidase-conjugated IgG3 (Mouse anti-human IgG3, Serotec) antibodies was used in the second step as secondary Ab. Results were expressed as Optical density (O.D.).

## HI ANTIBODIES BEFORE AND AFTER VIROSOMAL INFLUENZA VACCINE

Antigen	HIV-infected children (n = 24)			HC (n = 14)		
	Baseline	After 4 wks	After 24 wks	Baseline	After 4 wks	After 24 wks
<i>Seroconversion rates: N and (%)</i>						
A H3N2	-	13 (54.1)	-	-	10 (71.4)	-
A H1N1	-	17 (70.8)	-	-	13 (92.8)	-
B	-	17 (70.8)	-	-	12 (85.7)	-
<i>Seroprotection rates: N and (%)</i>						
A H3N2	4 (16.6)	19 (79.2)	13 (54.2)	4 (28.5)	13 (92.8)	10 (71.4)
A H1N1	9 (37.5) <sup>a</sup>	19 (79.2)	18 (75.0)	11 (78.6)	13 (92.8)	13 (92.8)
B	8 (33.3)	18 (75.0)	15 (62.5) <sup>b</sup>	6 (42.8)	13 (92.8)	13 (92.8)

a: P = 0.014; b: P = 0.04 vs. HC

## MEAN GMT (FOLD INCREASED) OF HI ANTIBODIES AFTER VIROSOMAL INFLUENZA VACCINE

Antigen	Baseline	Patients (n = 24)		Baseline	HC (n = 14)	
		After 4 wks	After 24 wks		After 4 wks	After 24 wks
A H3N2	10.29	44.90 (4.36) <sup>a,b</sup>	29.11 (2.83) <sup>a</sup>	12.81	76.14 (5.94) <sup>a</sup>	51.24 (4.00) <sup>a</sup>
A H1N1	20.00	146.72 (7.34) <sup>a,b</sup>	109.92 (5.50) <sup>a,b</sup>	44.16	609.08 (13.79) <sup>a</sup>	452.55 (10.25) <sup>a</sup>
B	16.82	77.72 (4.62) <sup>a</sup>	51.87 (3.08) <sup>a</sup>	16.41	107.67 (6.56) <sup>a</sup>	68.96 (4.20) <sup>a</sup>

a: P value < 0.01 vs. baseline b: P value < 0.01 vs. HC

## CONCLUSIONS

- Immunization with a single dose of virosome-adjuvated influenza vaccine results in the fulfilling of EMEA criteria for seroconversion, seroprotection, and GMT Ab increase in both HIV-infected children and in healthy controls.
- Despite these results, both humoral (mean GMT titers) and cell mediated (ELISPOT) influenza-specific responses are impaired in HIV-infected children.
- A significant increase of influenza-specific IgG3 /TH1-associated Ab is seen in healthy controls but not in HIV-infected children.
- Functional impairments of immune responses persist in HIV infection even in long term HAART-treated children with suppressed viremia and optimal immune recovery.

