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COMPREHENSIVE ANALYSIS OF B AND T CELL IMMUNE RESPONSES TO PANDEMIC pH1N1 MONOVALENT VACCINE IN HIV-INFECTED PREGNANT WOMEN

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Abstract

Background: Pregnant women have a high risk of severe influenza disease that can be mitigated by vaccination. Although HIV+ individuals have modest antibody responses to influenza vaccines, vaccination has been effective against disease, suggesting additional mechanisms of protection. We characterize the B and the T cell responses to pH1N1 vaccine and their determinants in HIV+ pregnant women.

Methods: 57 HIV+ pregnant women received two 30 µg doses of pH1N1 vaccine and had the following studies at entry and post-dose 1 and 2: IgG & IgA B cell ELISPOT; interferon (IFN)γ & granzyme B (GrB) T cell ELISPOT; hemagglutination inhibition (HAI) antibody titers; and B and T cell subsets by flow cytometry.

Results: Participants had mean± S.D. age= 27± 5 years, CD4= 521± 223 cells/µL and plasma $\log_{10}$ HIV RNA= 2.3± 0.9 copies/mL. All women were on antiretroviral treatment, including 51 on HAART.

HAI titers increased from median (interquartile range) of 20 (5, 40) at entry to 160 (40, 320) post-dose 1 and 2 (p=0.0001). ELISPOT-measured IgG antibody-secreting cells (ASC) increased from 6 (2, 28)/10^6 mononuclear cells (MNC) to 15 (4, 46) post-dose 1 (p=0.01) and 14 (6, 70) post-dose 2 (p=0.003). IgA ASC were low at entry [1 (0, 9)] and did not change after vaccination. ELISPOT-measured IFNγ spot-forming cells (SFC) decreased from 166 (38, 324)/ 10^6 MNC to 117 (40, 506) post-dose 1 and 76 (32, 336) post-dose 2 (p=0.06 post-dose 1 vs. 2). Vaccination did not change GrB SFC [entry of 48 (12, 258)].

IFNγ and GrB SFC inversely correlated with plasma HIV RNA at all time points (ρ≤ -0.35, p≤ 0.02) and IFNγ SFC positively correlated only with CD4% at entry (ρ= 0.31, p= 0.04). There were no significant correlations of ASC or HAI titers with HIV disease characteristics.

At all time points, IFNγ and GrB SFC were highly correlated with each other (ρ≥ 0.67, p< 0.0001). IFNγ SFC correlated with HAI titers at all time points (ρ≥ 0.29; p ≤ 0.06). IgG ASC did not correlate with IFNγ or GrB SFC or HAI titers. The table shows correlations of pH1N1 immune responses with the frequency of B and T cell subsets.

Conclusions: HIV+ pregnant women developed pH1N1 IgG, but not IgA, B cell immunologic memory after vaccination. pH1N1 T cell immunity was present at entry, did not increase after vaccination, and IFNγ SFC decreased post-dose 2. High Treg, Breg and activated T cells were associated with low T cell immunity, but high IL10+ Treg and Breg and activated B cells were associated with high B cell memory. High FOXP3+ Treg were associated with low B and T cell immunity.

Methods

•Two doses of Novartis influenza A (H1N1) 2009 Monovalent vaccine were administered as a 30mcg dose (given as two 15mcg intramuscular injections) at Day 0 and at Day 21 (+ 7 days).

•57 HIV-infected, pregnant women who participated in this 2-dose pH1N1 vaccine study had the following studies at entry and post-dose 1 and 2: IgG & IgA B cell ELISPOT; interferon (IFN)γ & granzyme B (GrB) T cell ELISPOT; hemagglutination inhibition (HAI) antibody titers; and B and T cell subsets by flow cytometry.

•Changes in ELISPOT and flow cytometry-measured parameters between different time points were assessed using Wilcoxon Matched Pairs Signed Ranks. Spearman correlation analyses were performed to test associations.

Characteristics of the Study Subjects

Variable	Total N=57
Race and Ethnicity : Black Latino	32 (56%) 25 (44%)
Age (yrs), Mean(Std Dev)	27 (5.5)
CD4+ %, Mean (Std Dev)	32 (10.0)
CD8+%, Mean (Std Dev)	45 (12.0)
Log 10 RNA, Mean (Std Dev)	2.3 (0.9)

pH1N1-Specific Humoral and Cellular Responses to Vaccinations and their Immunologic and Virologic Correlates

Figure 1. Kinetics of HAI Titers

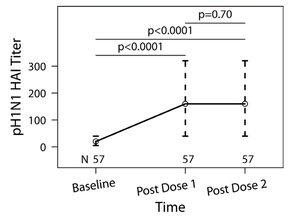


Figure 2. Kinetics of IgG ASC (Panel A) and IgA ASC (Panel B)

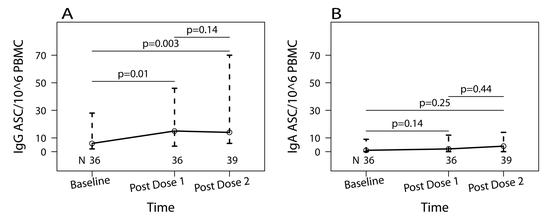
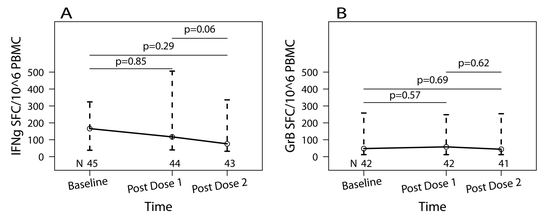


Figure 3. Kinetics of pH1N1 IFNγ SFC (Panel A) and GrB SFC (Panel B)



Legend: The points represent the median and the dotted lines extend to the 25th and 75th percentiles

Table 1. Correlations of HAI titers, B Cell and T Cell ELISPOT Measures over Time

Variable 1	Variable 2	Baseline		Post-dose 1		Post-dose 2	
		ρ (p value)	N	ρ (p value)	N	ρ (p value)	N
HAI Titers	IgG ASC	-0.11 (0.54)	36	-0.05 (0.77)	36	0.07 (0.68)	39
	INFγ SFC	0.35 (0.02)	45	0.32 (0.04)	44	0.29 (0.06)	43
INFγ SFC	IgG ASC	0.07 (0.71)	29	-0.12 (0.52)	31	0.15 (0.42)	32
	Granzyme B SFC	0.68 (<0.0001)	42	0.78 (<0.0001)	42	0.67 (<0.0001)	41
IgG ASC	IgA ASC	0.65 (<0.0001)	36	0.59 (0.0002)	36	0.50 (0.001)	39

Table 2. The Effect of Baseline HIV Disease Characteristics on Immune Responses

Variable 1	Variable 2 at baseline	Baseline		Post-dose 1		Post-dose 2	
		ρ (p value)	N	ρ (p value)	N	ρ (p value)	N
HAI Titers	CD4%	0.21 (0.12)	55	0.26 (0.06)	53	0.30 (0.03)	53
	CD8%	-0.19 (0.17)	55	-0.26 (0.06)	53	-0.34 (0.01)	53
	HIV RNA cp/mL	-0.44 (0.001)	55	-0.15 (0.30)	53	-0.24 (0.09)	53
IgG ASC	CD4%	0.04 (0.83)	36	-0.003 (0.99)	36	0.12 (0.45)	39
	CD8%	-0.04 (0.82)	36	-0.09 (0.62)	36	-0.03 (0.85)	39
	HIV RNA cp/mL	-0.04 (0.84)	36	-0.11 (0.53)	36	-0.14 (0.39)	39
INFγ SFC	CD4%	0.31 (0.04)	45	0.08 (0.62)	44	0.28 (0.07)	43
	CD8%	-0.23 (0.13)	45	-0.04 (0.81)	44	-0.27 (0.08)	43
	HIV RNA cp/mL	-0.49 (0.001)	45	-0.37 (0.01)	44	-0.43 (0.004)	43

Phenotypic Characterization of the B- and T-Cell Subsets

- The following B and T cell subsets were studied: CD19+CD21+CD27+ (resting memory), CD19+CD21+CD27-(naïve), CD19+CD21-CD27-CD20+ (tissue-like memory), CD19+CD21-CD27-CD20- (transitional),CD19+CD21-CD27+CD20- (plasmablasts), CD19+CD10+ (immature), CD19+CD10+CD27- (immature transitional), CD19+CD10+CD27+ (immature activated), CD19+IL10+ (Breg), CD19+CD25+ (Breg), CD4+CD39+ (Treg), CD4+HLADR+CD38+ (Tact), CD4+TGFB+ (Treg), CD4+IL10+ (Treg), CD4+FOXP3+ (Treg), CD4+FOXP3+CD25+ (Treg), CD8+CD39+ (Treg), CD8+HLADR+CD38+ (Tact), CD8+TGFB+ (Treg), CD8+IL10+ (Treg), CD8+FOXP3+ (Treg) and CD8+FOXP3+CD25+ (Treg).
- None of the subsets changed from baseline after vaccination.

Table 3. Correlations of Immune Responses with the Frequencies of B- and T-Cell Subsets

Variable	Subset (% of parent population)	Visit	ρ	P value
HAI Titers	CD8+HLADR+CD38+ activated T cells	Post-dose 1	-0.48	0.002
		Post-dose 2	-0.36	0.02
INFγ SFC	CD4+FOXP3+ regulatory T cells (Treg)	Entry	-0.37	0.04
	CD8+HLADR+CD38+ activated T cells	Post-dose 1	-0.40	0.03
	CD19+IL10+ regulatory B cells (Breg)	Post-dose 2	-0.36	0.05
IgG ASC	CD4+FOXP3+ Treg	Entry	-0.34	0.05
	CD4+CD25+FOXP3+ T reg	Entry	-0.35	0.046
	CD8+FOXP3+ Treg	Entry	-0.39	0.02
	CD19+CD21- CD27- transitional B cells	Post-dose 1	-0.36	0.03
	CD8+CD39+ T reg	Post-dose 1	0.42	0.02
	CD19+CD21- CD27+ activated B cells	Post-dose 2	0.35	0.04
	CD19+CD21- CD27- transitional B cells	Post-dose 2	-0.50	0.002

Conclusions

- HIV+ pregnant women had significant increases in HAI titers after vaccination.
- Women developed IgG but not IgA memory B cells after vaccination.
- Both antibody and memory B cell responses significantly increased after the 1st immunization with a double dose of pH1N1 vaccine, but not after the 2nd immunization.
- T cell immunity was present at entry and did not increase after vaccination.
- HAI titer increases after vaccination significantly correlated with IFNγ SFC suggesting an effect of Th1 on antibody responses to the vaccine.
- HAI titers after vaccination marginally correlated with the proportion of CD4+ cells and significantly, but negatively, correlated with the proportion of total and of activated CD8+ cells.
- pH1N1 IgG memory B cells were low at baseline in subjects with high proportions of Tregs. After vaccination IgG memory B cells were high in subjects with high proportions of activated B cells and low proportions of transitional B cells.
- High Th1 responses to pH1N1 correlated at all time points with low VL and after vaccination with low activated T cells and low Tregs.