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B AND T CELL SUBSETS AND IMMUNOGENICITY OF ROTAVIRUS VACCINE IN PHIV AND PHEU INFANTS

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ABSTRACT

Background IMPAACT P1072 was a double-blind placebo-controlled study of safety and immunogenicity of pentavalent rotavirus (RV5) vaccine in perinatally HIV-infected (PHIV) and exposed (PHEU) infants. This secondary analysis reports associations of HIV and RV5 with B and T cell subsets and correlations of subsets with RV5 antibody responses.
Design 89 infants (23 PHIV/19 PHEU vaccine- and 24 PHIV/ 23 PHEU placebo-recipients) had activated (act), inflammatory (inflam) and regulatory (reg) B/T cells and B cell differentiation measured before vaccination, 3wks post-dose 1 (PD1), and 2wks PD3. Immunogenicity was measured by anti-RV5 IgA and anti-G1-4 and P IgG. Spearman correlations ($|\rho| \geq 0.2$) were used to identify associations. Area under the curve (AUC) was calculated for markers ($\log_{10}$ scale) using predicted values from mixed random effects models.
Results At entry: PHIV had median HIV viral load (VL) of 33,500 c/mL and 28% CD4+ cells, and 89% had initiated cART. PHEU and PHIV did not differ significantly (p>0.05) in B/T cell subset distribution. In PHIV, VL positively correlated with act CD8+CD38+HLADR+%, CD19+IL21r+%, CD19+BAFFr+% and immature CD19+CD10+%; cART duration negatively correlated with inflam CD4+/CD8+CD17+%. In PHIV and PHEU, CD4+% and counts positively correlated with reg CD8+CD25+FOXP3+%. Among lymphocyte subsets, reg CD4+/CD8+/CD19+IL10+% and CD4+/CD8+/CD19+TGFb+% in general positively correlated with inflam CD4+/CD8+IL17+%; and act CD4+/CD8+CD38+HLADR+% positively correlated with immature CD19+CD10+%.
Vaccination and HIV status were not significantly associated with differential changes in B/T cell subsets except for reg CD4+IL10+%, which increased in PHIV vaccinees and decreased in PHEU and PHIV placebo recipients.
IgA and/or IgG responses to RV5 positively correlated with CD4+% and/or counts and with reg CD4+/CD8+CD25+FOXP3+%; but negatively correlated with reg CD4+IL10+%, CD4+TGFb+% and inflam CD4+IL17+% (Figure).
Conclusions We did not find reg, inflam or act B/T-cell differences between PHEU and PHIV. Higher inflam, IL10+ and TGFb+ Tregs were associated with lower responses to RV5, while CD25+FOXP3+ Tregs were associated with higher responses. IL10+ and TGFb+ B/Tregs were associated with higher Tinflam, while CD25+FOXP3+ Tregs were associated with higher CD4+ cells. This is the 1st demonstration that phenotypically distinct Tregs differ in the way they associate with immune responses to vaccination.

BACKGROUND

- B and T cell compartments have altered subset distribution and functionality in HIV-infected individuals
- It is not clear how these abnormalities affect the host immune responses to infectious agents or vaccines
- We investigated this relationship by measuring B and T cell subsets in perinatally HIV-infected (PHIV) and exposed (PHEU) infants enrolled in IMPAACT P1072, a double-blind, placebo-controlled safety and immunogenicity study of the pentavalent rotavirus vaccine (RV5) in 76 PHIV on cART and 126 PHEU
  - PHIV and PHEU who received RV5 had similar IgG and IgA antibody titers against the RV5 components and significantly higher titers compared with placebo recipients
- In this secondary analysis we present:
  - the effect of HIV status and vaccination on the frequencies of regulatory, activated and inflammatory T cells, and of memory, exhausted and immature B cells
  - correlations among the T and B cell subsets
  - correlations of regulatory T cells (Treg) and select inflammatory and B cell subsets with antibody responses to vaccination.

METHODS

Study design: Infants 2 -<15 weeks at screening were determined to be PHEU or PHIV and randomized to receive 3 doses of RV5 or placebo according to the recommended schedule. Participants were followed until 6 weeks after the last dose. Blood was collected at entry, 21 (±7) days after the 1st dose of vaccine and 14 (11-21) and/or 42 (28-70) days after the 3rd dose. This analysis included infants who received 3 doses of vaccine per-protocol, had sufficient peripheral blood mononuclear cells (PBMC) for flow cytometry assessment at ≥3 of the 4 possible blood collection time points, collected within pre-specified time intervals.

Antibody measurements: to RV5 were measured on serum obtained at baseline and 14 or 42 days after the 3rd dose of vaccine and included neutralizing antibodies (SNA) targeting the viral capsid proteins G1, G2, G3, G4 and P1A, and IgA antibodies that recognize RV5 epitopes in RV5-infected fibroblasts.

Cytokine measurements: IFNγ, IL-2, IL-6 and IL-10 were measured in plasma collected at study entry (pre-vaccination) and at 21 days after the 1st vaccine dose using the ultrasensitive 10-plex human cytokine kit (V-Plex proinflammatory kit) from Meso-Scale Discoveries (MSD, Rockville, MD) according to the manufacturer's directions.

Flow cytometric analysis: B- and T-cell subsets were enumerated in freshly thawed cryopreserved PBMCs. After washing and counting viable cells, PBMCs were surface-stained with the following conjugated mAbs: anti-CD3-AF488 (Biolegend; clone HIT3a), anti-CD8-APC/AF750 (Invitrogen; clone 3B5), anti-CD8-PE/Cy7 (Invitrogen; clone 3B5), anti-CD19-PE/Cy5 (BD Biosciences; clone HIB19), anti-CD19-PE/Cy7 (eBioscience; clone HIB19), anti-CD-10-APC/Cy7 (Biolegend; clone HI10a), anti-CD25-APC/Cy7 (BD Biosciences; clone M-A251), CD21-PE/Cy5 (BD Biosciences; clone B-ly4), CD38-PE (Invitrogen; clone HIT2), CD27-APC (BD Biosciences; clone L128). Cells were fixed and permeabilized using a Fixation/Permeabilization kit (eBioscience), and stained with HLA-DR-PE/Cy5 (BD Biosciences; clone G46-6), IL-10-APC (BD Biosciences; clone JES3-19F1), IL-17-PE/Cy7 (Biolegend; clone BL168), FoxP3-APC (eBioscience; clone PCH101), IL-2r-PE (BD Biosciences; clone 17A12), TGFb-PE (Cedarlane; clone TB21) BAFFr-FITC (BD Biosciences; clone 11C1) and analyzed with Guava easyCyte 8HT and FlowJo (Treestar). Subsets were expressed as percentages of the parent CD4+, CD8+ and CD19+ cell populations.

Statistical analysis: Distributions of biomarkers at entry were compared by HIV-1 status using Wilcoxon rank sum tests. Spearman correlations were calculated among biomarkers and other participant characteristics. To assess changes in marker levels over time, mixed models with random intercepts and slopes were fit on the $\log_{10}$-transformed measurements to determine whether the slopes were associated with HIV-1 status or vaccine administration. To reflect marker level at entry and over time, area-under-the-curve (AUC) was calculated using individual-level effects from the mixed models, and the AUCs correlated with antibody responses to RV5 in vaccine recipients. Because of the large number of biomarkers and exploratory nature of the analyses, results should be viewed as hypothesis-generating in nature.

RESULTS

Table 1: Characteristics at study entry by HIV status and whether included in flow analyses

		HEU: Flow (N=42)	HEU: No flow (N=84)	HIV+: Flow (N=47)	HIV+: No flow (N=29)
Country	Botswana	11 (26%)	26 (31%)	17 (36%)	16 (55%)
	Tanzania	5 (12%)	2 (2%)	5 (11%)	1 (3%)
	Zambia	4 (10%)	4 (5%)	3 (6%)	3 (10%)
	Zimbabwe	22 (52%)	52 (62%)	22 (47%)	9 (31%)
Sex	Male	15 (36%)	44 (52%)	20 (43%)	15 (52%)
	Female	27 (64%)	40 (48%)	27 (57%)	14 (48%)
Age at randomization (days)	Median (Min, Max)	92 (52, 101)	78 (28, 103)	93 (61, 104)	93 (39, 104)
Ever breast fed	Yes	23 (55%)	56 (67%)	30 (64%)	18 (62%)
	No	19 (45%)	28 (33%)	17 (36%)	11 (38%)
PMTCT [1]	No	8 (19%)	5 (6%)	14 (30%)	11 (38%)
	Yes	34 (81%)	79 (94%)	33 (70%)	18 (62%)
Mother on ARVs [2]	No	14 (33%)	35 (42%)	38 (81%)	24 (83%)
	Yes	28 (67%)	49 (58%)	9 (19%)	5 (17%)
ARV (days) [3]	Median (Min, Max)			6 (0, 49)	1 (0, 50)
WHO weight-for-age z-score	Median (Q1, Q3)	-0.7 (-1.4, -0.1)	-0.6 (-1.3, 0.0)	-1.4 (-2.7, -0.2)	-1.5 (-2.4, -0.5)
Screening CD4%	Median (Min, Max)	36 (20, 66)	40 (19, 63)	28 (7, 58)	30 (15, 47)
	<15%	0 (0%)	0 (0%)	3 (6%)	0 (0%)
	15% - <20%	0 (0%)	1 (1%)	4 (9%)	4 (14%)
	≥ 20%	42 (100%)	83 (99%)	40 (85%)	25 (86%)
Entry HIV-1 RNA (copies/ml)	Median			33,491	204,428
	< 10K			15 (32%)	7 (28%)
	10K - <100K			14 (30%)	4 (16%)
	100K - <750K			10 (21%)	5 (20%)
	≥ 750K			8 (17%)	9 (36%)
	Not measured			0	4

[1] PMTCT = Prevention of mother-to-child transmission
[2] ARV = Antiretroviral therapy
[3] Six infants were not on ARVs when they received the first study vaccination

Table 2: B and T cell phenotypic characteristics and relevant cytokine concentrations at study entry

		PHEU				PHIV			
Assay	Wilcoxon pvalue	N	Median	25th	75th	N	Median	25th	75th
CD4+CD38+HLADR+ (activated T cells)	0.87	38	7.1	5.0	10.8	39	7.9	4.3	10.8
CD4+IL17+ (mucosal effector T cells)	0.31	40	2.8	1.3	5.9	42	3.6	1.6	7.5
CD4+CD25+FOXP3+ (regulatory T cells)	0.43	38	0.1	0.0	0.2	39	0.1	0.0	0.2
CD4+IL10+ (regulatory T cells)	0.77	40	1.9	0.7	3.2	42	1.9	1.0	3.2
CD4+TGFb+ (regulatory T cells)	0.38	40	2.8	1.6	4.3	42	2.7	1.8	7.9
CD8+CD38+HLADR+ (activated T cells)	0.62	37	14.4	10.8	21.6	38	13.1	10.4	19.3
CD8+IL17+ (mucosal effector T cells)	0.71	41	4.9	2.4	12.0	43	5.6	3.0	11.7
CD8+CD25+FOXP3+ (regulatory T cells)	0.21	37	0.3	0.1	0.5	38	0.2	0.1	0.4
CD8+IL10+ (regulatory T cells)	0.85	41	1.7	1.2	4.6	43	1.7	1.2	5.3
CD8+TGFb+ (regulatory T cells)	0.40	41	3.2	1.8	5.7	43	4.1	1.8	10.6
CD19+CD10+ (immature B cells)	0.93	41	10.0	5.8	15.7	42	9.0	5.0	17.2
CD19+C10-CD21+CD27- (naive B cells)	0.15	41	0.3	0.1	1.2	42	0.2	0.1	0.4
CD19+C10-CD21+CD27+ (memory B cells)	0.07	41	0.8	0.5	1.8	42	1.2	0.6	3.1
CD19+C10-CD21-CD27- (exhausted B cells)	0.35	41	94.4	89.2	98.3	42	92.4	86.6	97.5
CD19+IL21r+ (B cells)	0.29	41	6.3	5.5	8.6	42	5.5	4.3	8.9
CD19+BAFFr+ (B cells)	0.08	41	4.8	2.9	7.5	42	3.7	1.3	6.2
CD19+IL10+ (regulatory B cells)	0.18	41	2.2	1.1	3.0	39	2.6	1.7	3.5
CD19+TGFb+ (regulatory B cells)	0.59	41	4.2	2.0	16.6	39	3.8	1.4	11.2
IL-2 (pleiotropic)	0.001	41	0.3	0.2	0.4	46	0.5	0.3	0.8
IL-6 (inflammatory)	0.043	41	1.4	0.6	2.9	46	2.7	0.7	10.2
IFN gamma (pro-inflammatory)	0.014	41	18.9	12.0	28.6	46	29.2	16.0	55.7
IL-10 (regulatory)	<0.001	41	2.0	1.7	2.7	46	3.2	2.2	6.8

Figure 1: Heatmap of Spearman correlations (p-values) of entry biomarkers with other entry characteristics (PHIV and PHEU)

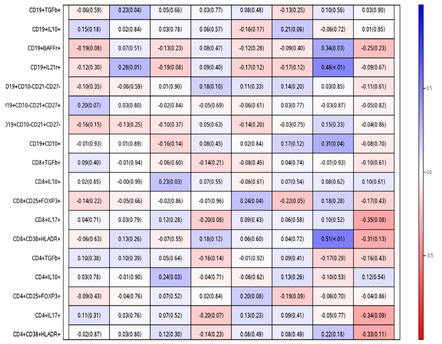


Figure 2: Heatmap of Spearman correlations among entry biomarkers (PHIV and PHEU)

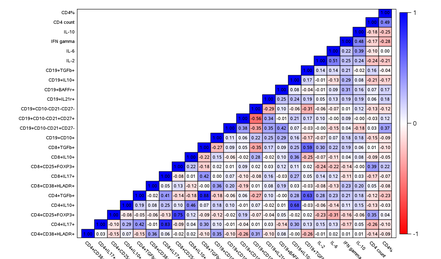
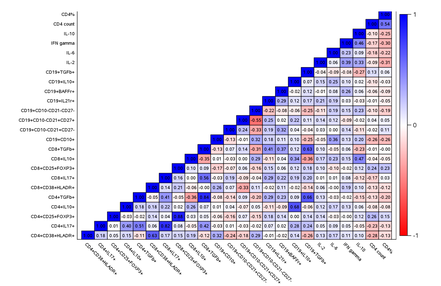


Figure 3: Heatmap of Spearman correlations among biomarker AUCs (PHIV and PHEU)

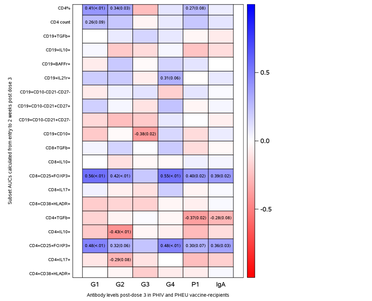


Changes in biomarkers with vaccination or HIV status

In mixed models of biomarker slopes with vaccine, HIV status and their interaction:

- CD4+IL10+% regulatory cells increased significantly in PHIV vaccine-recipients only (p=0.013) compared to PHEU and PHIV on placebo
- no other biomarkers changed over time

Figure 4: Spearman correlations of T and B-cell subsets and antibody responses to RV5



CONCLUSIONS

1. PHIV on cART and PHEU did not differ with respect to B cell differentiation and abundance of inflammatory and regulatory B and T cell subsets. This observation may be relevant to the immune deficit and other abnormalities observed in PHEU.

1. Higher antibody responses to RV5 were associated with:
   a. lower proportions of regulatory T cells that produce IL10 or TGFβ
   b. lower proportions of Th17 inflammatory T cells (which positively correlated with IL10+ and/or TGFβ+ regulatory T cells)
   c. lower proportions of CD10+ immature B cells
   d. higher proportions of CD25+FOXP3+ regulatory T cells
   e. higher proportions of B cells expressing the IL21 receptor
   f. higher proportions and absolute numbers of CD4+ T cells

2. Regulatory IL10+ and TGFβ+ B and T cell subsets differed from CD25+FOXP3+ regulatory T cells with respect to their associations with antibody responses to RV5 and their associations with CD4+ counts and/or percentages, inflammatory T cells and immature or exhausted B cells:
   a. IL10+ and TGFβ+ subsets correlated with high levels of inflammation, immaturity and exhaustion
   b. CD25+FOXP3+ regulatory T cells correlated with immune preservation.

This novel observation has high relevance for Treg therapeutic applications.

Acknowledgements

We gratefully acknowledge the contributions of the site investigators and staff who conducted the P1072 study and the children and caregivers who participated in the study.