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Regulatory T-cell Contraction in Early Pregnancy Associated with HIV Infection and Preterm Birth

Katelyn J. Rittenhouse[1], Humphrey Mwape[2], Kristina De Paris[1], John Mwale[2], Gabriel Chipili[2], Joan T. Price[1,2], Elizabeth Stringer[1], Bellington Vwalika[2,3], Jeffrey S. A. Stringer[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]University of North Carolina Global Projects Zambia, Lusaka, Lusaka, Zambia, [3]University of Zambia School of Medicine, Lusaka, Lusaka, Zambia.

Background

- HIV-infection and its treatment have been associated with increased risk of preterm birth (PTB).
- A peripheral expansion of regulatory T cells (Tregs) during the 2nd trimester has been proposed as a mechanism for maternal tolerance of the fetal allograft. However, recent studies applying more stringent immunophenotyping methods specific for viable, suppressive Tregs demonstrate that this population remains stable and does not expand during pregnancy.
- To date, few studies have evaluated Treg frequencies in HIV-infected compared to uninfected gravidas, or characterized Treg frequencies among pregnant women in sub-Saharan Africa.

Objectives

- To quantify Treg populations in the first and second trimesters of pregnancy in an ongoing sub-Saharan African antenatal cohort with high prevalence of HIV and PTB.
- To investigate whether altered peripheral Treg frequencies are associated with HIV and/or PTB.

Methods

- This project was a sub-study of the Zambia Preterm Birth Prevention Study (ZAPPS; ClinicalTrials.gov Identifier: NCT02738892). ZAPPS is a prospective cohort at the Women and Newborn Hospital of the University Teaching Hospital in Lusaka, Zambia.
  - Pregnant women ≥18 years with a viable singleton or twin pregnancy enrolled at ≤24 weeks of gestation, confirmed by ultrasound, are followed through pregnancy, delivery, and up to 42 days postpartum.
  - Written informed consent for collection of detailed medical and obstetrical history, biological specimens, physical exams, routine antenatal tests (e.g. HIV, syphilis, malaria) and ultrasound assessments is obtained prior to study enrollment.
  - All ZAPPS participants are screened for HIV upon presentation for antenatal care with SD Bioline 3.0 rapid test (SD Biostandard Diagnostics, India). Positive cases are confirmed with Determine HIV-1/2 Ag/Ab Combo test (Alere Inc., Waltham, MA). HIV-1 viral loads are assessed in HIV-infected participants at the first study visit using Abbott RealTime HIV-1 assay (Abbott Laboratories, Abbott Park, IL).
- Peripheral venous blood was collected in sodium heparinized vacutainers at enrollment and 24-week antenatal visits between May 2017 and January 2018. Specimens were processed within 24 hours of collection using the Human Treg Staining Kit (eBioscience, San Diego, CA). Tregs were identified using CD4+CD25+CD127lowFoxP3+ immunophenotype (Figure 1).
- Paired and unpaired specimens were analyzed (Figure 2) in this sub-study. Paired and unpaired t-tests were used to compare log-transformed Treg frequencies (percent of parent CD4+ cells) by HIV serostatus. Additionally, paired Treg specimens were defined as expanding or contracting and assessed by HIV serostatus and PTB using chi-square test.
- To account for multiple comparisons, we set our threshold for statistical significance at 1% (i.e. p≤0.01) and refer herein to p-values >0.01 and <0.05 as a "trend."

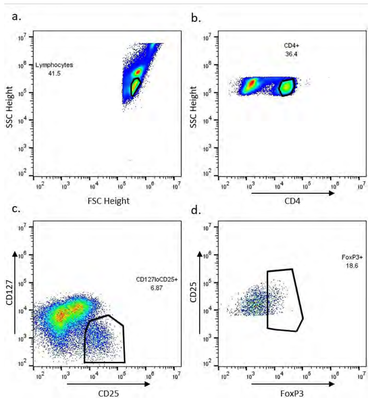


Figure 1: Gating strategy for Treg analysis: (**a**) Lymphocytes are gated using forward scatter vs. side scatter characteristics. (**b**) CD4+ lymphocytes are gated. (**c**) CD4+ lymphocytes are analyzed for CD25 and CD127 expression, number represents the percentage of CD4+ lymphocytes that are CD25+CD127low. (**d**) CD4+CD25+CD127low cells are analyzed for FoxP3+ expression, number represents the percentage of CD4+CD25+CD127low cells that are FoxP3+.

Results

Figure 2. Distribution of participants and specimens (paired and unpaired) included in sub-study

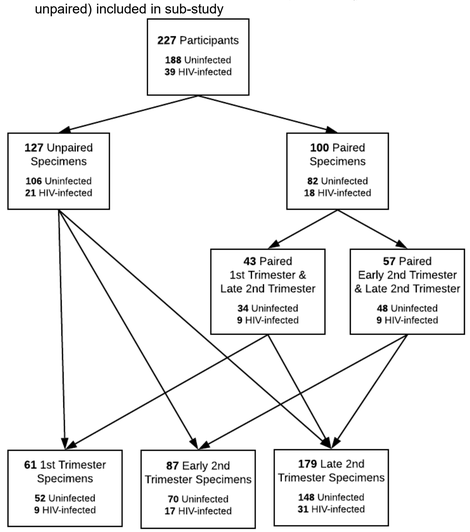


Table 1: Baseline characteristics of participants by gestational age group

Baseline Characteristic	First Trimester 7-14 weeks: Uninfected N (%) or median (IQR)	First Trimester 7-14 weeks: HIV-infected N (%) or median (IQR)	First Trimester 7-14 weeks: p-value	Early Second Trimester 14-22 weeks: Uninfected N (%) or median (IQR)	Early Second Trimester 14-22 weeks: HIV-infected N (%) or median (IQR)	Early Second Trimester 14-22 weeks: p-value	Late Second Trimester 22-28 weeks: Uninfected N (%) or median (IQR)	Late Second Trimester 22-28 weeks: HIV-infected N (%) or median (IQR)	Late Second Trimester 22-28 weeks: p-value
Population (n)	52	9		70	17		148	31	
EGA at specimen collection, wks	11 (10, 12)	11 (10, 12)	ns	17 (16, 18)	17 (16, 19)	ns	25 (24, 25)	24 (24, 25)	ns
Age, years	27 (24, 32)	34 (30, 35)	<0.01	27 (23, 34)	31 (29, 35)	0.10	27 (23, 32)	30 (29, 34)	0.02
BMI, median kg/m²	23 (21, 29)	27 (23, 31)	ns	24 (22, 29)	23 (21, 26)	ns	24 (21, 28)	24 (21, 29)	ns
Nulliparous	24 (49%)	0 (0%)	<0.01	24 (35%)	1 (6%)	0.02	57 (41%)	3 (10%)	<0.01
Prior Preterm Birth	15 (31%)	6 (67%)	0.04	22 (32%)	9 (53%)	0.12	46 (33%)	19 (63%)	<0.01
Twin Gestation	2 (4%)	0 (0%)	ns	1 (1%)	0 (0%)	ns	7 (5%)	0 (0%)	ns
Bacteriuria	3 (6%)	1 (11%)	ns	3 (4%)	1 (6%)	ns	8 (5%)	4 (13%)	ns
Malaria	0 (0%)	0 (0%)	ns	0 (0%)	0 (0%)	ns	0 (0%)	1 (3%)	ns
Syphilis	0 (0%)	1 (11%)	0.02	0 (0%)	1 (6%)	0.04	3 (2%)	4 (13%)	<0.01
Detectable Viral Load	na	2 (22%)		na	6 (35%)		na	11 (38%)	

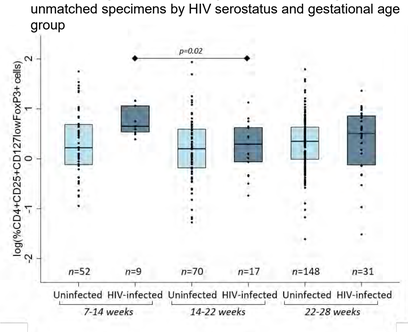
Baseline characteristics collected at enrollment (<24 gestational weeks)
IQR: interquartile range; BMI: body mass index; EGA: estimated gestational age; HAART: highly active antiretroviral therapy; ns: not significant
p-values calculated by $\chi 2$ for categorical and Wilcoxon rank-sum test for continuous comparisons

Table 2: Proportion of paired specimens with Treg contraction by HIV serostatus and gestational age at delivery groups

	1st Trimester & Late 2nd Trimester	Early 2nd Trimester & Late 2nd Trimester
HIV Serostatus Group		
Uninfected	15/34 (44%)	27/48 (56%)
HIV-infected	8/9 (89%)	5/9 (54%)
	p=0.01	p=0.93
Gestational Age at Delivery Group		
Term Birth	11/22 (50%)	19/31 (61%)
Preterm Birth	7/7 (100%)	6/12 (50%)
	p=0.01	p=0.50

Preterm birth defined as parturition prior to 37 gestational weeks
p-values calculated by $\chi 2$

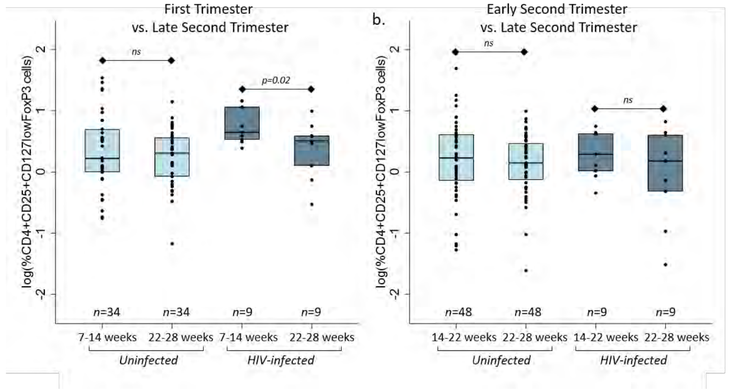
Figure 3. Log-transformed Treg frequencies among 327 unmatched specimens by HIV serostatus and gestational age group



Sensitivity analyses:

- When excluding the second of two paired specimens to control for potential correlation between datapoints, no new associations were observed in our unmatched analysis, but the trend toward decreased Treg frequency in HIV-infected women was again observed between first trimester and early second trimester specimens.
- When excluding 28 specimens from women with presumed concurrent infection at enrollment (bacteriuria, malaria, and syphilis), the previously identified trend toward contraction in HIV-infected women trend persisted (p=0.02).
- In analyses of HIV-infected women by viral load (VL) sub-groups, the mean log-transformed Treg frequency was observed to be decreasing in both women with suppressed VL (1st trimester: n=7, mean: 0.77; early 2nd trimester: n=11, mean: 0.36; p=0.07) and unsuppressed VL (1st trimester: n=2, mean: 0.70; early 2nd trimester: n=6, mean: 0.02; p=0.12). However neither decrease reached statistical significance or tendency.

Figure 4. Change in log-transformed Treg frequencies by HIV serostatus between (a.) 7-14 week and 22-28 week paired specimens and between (b.) 14-22 week and 22-28 week paired specimens



Conclusions

- Exploratory data from our African cohort, established specifically to study PTB, demonstrate a Treg contraction between the 1st and 2nd trimesters of pregnancy in HIV-infected women that is also associated with PTB.
- This finding suggests that Treg populations may play a systemic role in HIV-associated PTB and warrants further investigation. Future research should include analysis of Treg subsets and include functional assays to better understand specific alterations in peripheral circulation in HIV-infected women as well as in PTB.
- We are currently collecting additional Treg data in HIV-infected women with known preconceptional ART exposure in an ongoing study in Lusaka, Zambia to better characterize the interaction between preconceptional and postconceptional ART and peripheral Treg frequencies in HIV-infected women during pregnancy.

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