Mucosal-homing Invariant NKT Cells are Elevated in HIV-exposed Seroconverters Prior to Infection

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Abstract

Background: Studies in both humans and macaques have identified markers of HIV susceptibility such as the gut-homing integrin α4β7, whose expression in memory CD4+ T cells increases in the context of HIV infection and is associated with increased susceptibility to acquisition[1,2,3]. However, the phenotypic characteristics of unconventional T cells in the context of HIV infection susceptibility are not fully understood. We therefore sought to characterize unconventional T cell phenotypes in 25 HIV-exposed seroconverted (HESC) individuals prior to seroconversion and compare them to HIV-exposed seronegative (HESN) individuals.

Methods: RV217 is a prospective natural-history study which recruited individuals at high risk for HIV acquisition[3]. PBMC from the RV217 cohort were used to investigate T cell phenotypes in 25 HESC individuals prior to seroconversion. Each HESC study participant was matched for age, gender, and risk behavior with 3 HESN individuals for comparative analysis. A multicolor flow cytometry panel was developed to investigate mucosal associated invariant T (MAIT), invariant natural killer T (iNKT), gamma delta T, and conventional CD4+ T cell phenotypes prior to HIV seroconversion.

Results: Our analysis showed increased frequency of total α4β7 expressing CD4+ memory T cells in HESC individuals (%α4β7+, p=0.0486; %α4β7^{high}, p=0.0009) compared to HESN individuals. Additionally, our study shows increased frequency of α4β7 expressing iNKT cells in HESC individuals compared to HESN individuals (%α4β7+, p=0.0304). To further characterize α4β7 expressing iNKT cells, we analyzed α4β7 expression in major iNKT cell subsets: CD4+ iNKT cells, CD8+ iNKT cells and CD4-CD8- (DN) iNKT cells. Our results reveal that α4β7 expression in HESC individuals is increased in CD4+ iNKT cells (p=0.0342) and not CD8+ iNKT (p=0.5424) or DN iNKT cells (p=0.1817) compared to HESN individuals. No differences between the groups were found in MAIT and gamma delta T cells.

References

1. Cicala, C., Martinelli, E., McNally, J. P., Goode, D. J., Gopaul, R., Hiatt, J., ... & Arthos, J. (2009). The integrin α4β7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. *Proceedings of the National Academy of Sciences*, *106*(49), 20877-20882.
2. McKinnon, L. R., Nyanga, B., Chege, D., Izulla, P., Kimani, M., Huibner, S., ... & Kaul, R. (2011). Characterization of a human cervical CD4+ T cell subset coexpressing multiple markers of HIV susceptibility. *The Journal of Immunology*, *187*(11), 6032-6042.
3. Sivro, Aida, et al. "Integrin α4β7 expression on peripheral blood CD4+ T cells predicts HIV acquisition and disease progression outcomes." Science translational medicine 10.425 (2018): eaam6354.
4. Robb, M.L. et al. Prospective study of acute HIV-1 infection in adults in East Africa and Thailand. N. Engl.J. Med. 2016; 374, 2120–2130

Methods

1. Prospective Therapy-naïve cohort RV217:

- 3,224 East Africa & Thailand participants
- 2,276 volunteers at high-risk for HIV-1
- Participants tested 2X weekly for early capture
- 112 participants acquired acutely HIV-1

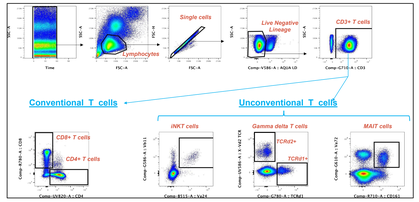
	HESC N=25	HESN N=75
Female n(%)	1(4%)	3 (4%)
Male n(%)	24 (96%)	72 (96%)
Syphilis	2/25	4/50
Hepatitis B Ag	0/25	2/24
Days to first HIV+ (Median, 25%-75% IQR)	79 (48-120)	NA

2. Participants Demographical Characteristics:

- HESC & HESN were paired 1:3, related to age, gender & risk behavior. Pre-HIV acquisition cryopreserved PBMCs were used for flow cytometry analysis.

3. Conventional and Unconventional T cell phenotyping

- A 29-parameter flow cytometry panel was used to quantify conventional and unconventional T cell frequency and phenotype.



Channel	Fluorochrome Label	Innate T cells markers	Markers Function
B515	BB515	TCR Va24	iNKT cells
B610	BB630-P	CCR6	Migration marker
B670	BB660-P2	CD69	Activation marker
B780	BB790	CXCR5	Migration marker
R670	APC	a4b7	Gut homing marker
R710	R718	CD161	MAIT cells
R780	APC Cy 7	CD8	T cell lineage
V450	BV421	MR1 Tetramer	MAIT cells
V474	BV480	HLA-DR	Activation marker
V586	BV570	Aqua	Exclusion of Dead cells
V586	BV570	CD19	Non-innate Cell lineage exclusion
V586	BV570	CD14	Non-innate Cell lineage exclusion
V610	BV605	Granzyme B	Activation marker
V670	BV650	CCR7	Migration marker
V710	BV711	pan TCR gamma delta	Gamma delta T cells
V780	BV786	CD45RO	Differentiation marker
V740	BV750	Ki67	Proliferation marker
UV379	BUV395	CD57	Maturation marker
UV515	BUV496	CD16	NK cells
UV586	BUV563	TCR Vd2	Gamma delta T cells
UV610	BUV615-P	CD56	NK cells
UV670	BUV661	PD-1	Activation/exhaustion marker
UV740	BUV737	CD38	Activation marker
UV820	BUV805	CD4	T cell lineage
G586	PE	TCR Vb11	iNKT cells
G610	PE-Dazzle594	TCR Va7.2	MAIT cells
G710	PE Cy 5.5	CD3	T cell lineage
G780	PE Cy 7	TCR Vd1	Gamma delta T cells
G670	PE Cy 5	T-bet	Transcription factor

Results

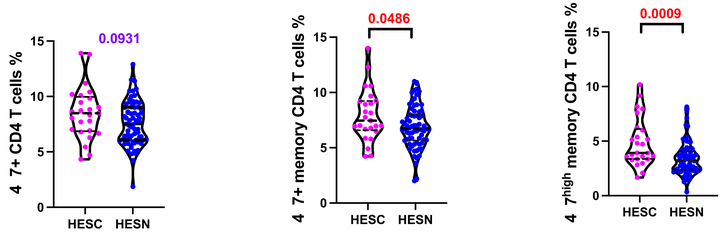


Figure 1: HESC have increased expression of α4β7 in memory CD4+ T cells

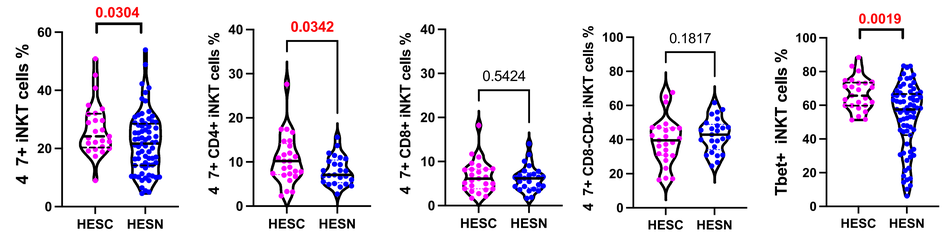
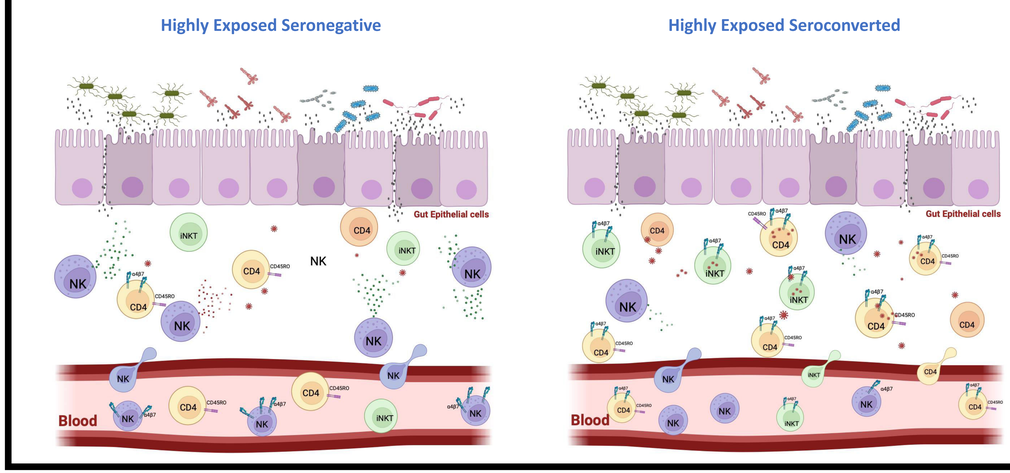


Figure 2: HESC have increased expression of α4β7 and T-bet in iNKT cells

Conclusions

Our study supports the existing role of α4β7high CD4+ memory T cells in HIV susceptibility and identifies a potential role for gut homing α4β7+ CD4+ iNKT cells in HIV susceptibility in the context of individuals at high risk for HIV acquisition.



Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.