A quiescent NK cell phenotype with gut homing potential in HIV-1 exposed seronegative individuals

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Abstract

Background: The mechanisms responsible for susceptibility to HIV acquisition are poorly defined and most likely are multifactorial. The study of HIV-exposed seronegative (HESN) and seroconverted (HESC) brings important information about susceptibility to HIV acquisition and opens doors for new preventive and therapeutic strategies**.** NK cell function has been been linked with HIV protection. Increased NK cytotoxicity and activation have been correlated with protection in HESN, suggesting the involvement of the innate immune responses in decreasing the risk of HIV-1 acquisition[1-2]. However, others have shown a lower expression of activation and exhaustion markers on NK cells in HESN[3]. These discrepancies are because HESN are compared to cohorts of healthy donors with very low risks of exposure or to people living with HIV. Here we compare circulating NK cell phenotype in HESN to HESC participants with similar risk behavior, age and gender, prior to HIV-1 acquisition[4].

Methods: Specimens from participants in the RV217 Early Capture HIV Cohort (ECHO)[4] were used to investigate NK cell in 25 HESC and 75 HESN participants. For our analysis, pre-infection samples were collected for each HESC individual and were matched for collection time and location, age, gender, and risk behavior with 3 HESN. A 26-parameter flow cytometry panel was used to quantify NK cell frequency and phenotype. A pre-infection time point, between 28-365 days prior to first HIV positive test was chosen for the HESCs.

Results: Compared with HESC individuals, NK cells from HESN participants were significantly less activated, as measured by the expression of HLA-DR (%, p=0.003 & MFI, *p*=0.001) and had a tendency for lower expression of the proliferation marker Ki67. Moreover, NK cells from HESN showed a higher potential to migrate to the gut measured by the expression of $\alpha 4\beta 7$ (%, *p*=0.010 & MFI, *p*=0.015) and a lower expression of the inhibitory receptor ILT-2 (%, *p*=0.035 & MFI, p=0.023) compared to HESC participants. No differences were observed related to total NK cell frequency, distribution of the CD56hi immature, CD56dim mature, or CD56low NK cell subsets. No associations were found between NK cell phenotype and peak or set point viral load following acquisition in the HESC participants.

Conclusions: Our preliminary data show significant differences in NK cells between HESN and HESC. The increased activation and expression of the inhibitory receptor ILT2, together with a lower expression of gut homing marker $\alpha 4\beta 7$, suggest that NK cells from HESC participants might have an exhausted and dysfunctional phenotype with lower ability to migrate to the gut mucosa. It is possible that this may increase the risk of HIV-1 acquisition relative to a more beneficial quiescent NK cell profile as observed in the HESN participants.

References

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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

2- Participants Demographical Characteristics:

- HESC & HESN have been paired 1:3, related to age, gender & risk behavior. Pre-infection cryopreserved PBMCs were used for NK cells analysis.

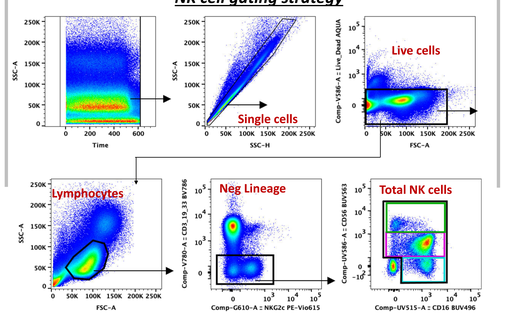
	HESC N=25	HESN N=75
Female n(%)	1(4%)	3 (4%)
Male n(%)	24 (96%)	72 (96%)
Syphilis	2/25	4/50
Hepatitis B Ag	0/25	2/24
Days to first HIV+ (Median, 25%-75% IQR)	79 (48-120)	NA

3- NK cell phenotyping

- A 26-parameter flow cytometry panel was used to quantify NK cell frequency and phenotype[5].

Channel	Fluorochrome Label	NK cells markers	Markers Function
B515	FITC	FcRg	NK cell subset
B710	BB700	CD158a/h/g (KIR2DL1/S1/S3/S5)	Activating and inhibiting receptors
	BB700	CD158e1 (KIR3DL1)	Activating and inhibiting receptors
B780	BB790-P	NKG2D	Activating receptor
R670	APC	a4b7	Gut homing marker
R710	R718	NKp30	Activating receptor
R780	APC-Vio770	CD328 (Siglec 7)	Inhibiting receptor
V450	BV421	KLRG1	Inhibiting receptor
V474	BV480	HLA-DR	Activating receptor
V586	Aqua	Live/Dead	Exclusion of dead cells
V670	BV650	NKp80	Activating receptor
V710	BV711	NKp46	Activating receptor
V740	BV750	Ki67	Proliferation marker
V780	BV786	CD33	Non-NK cell lineage exclusion
V780	BV786	CD19	Non-NK cell lineage exclusion
V780	BV786	CD3	Non-NK cell lineage exclusion
UV379	BUV395	CD57	Maturation marker
UV515	BUV496	CD16	NK cell subset
UV586	BUV563	CD56	NK cell subset
UV615	BUV615	ILT2	Inhibiting receptor
UV670	BUV661	PD-1	Activation/Exhaustion marker
UV740	BUV737	CD38	Activating receptor
UV820	BUV805	CD94	Activating and inhibiting receptors
G586	PE	EOMES	Transcription factor
G610	PE-Vio615	NKG2C	Activating receptor
G670	PE Cy 5	T-Bet	Transcription factor
G780	PE-Vio770	NKG2A (CD159a)	Inhibiting receptor

NK cell gating strategy



Results

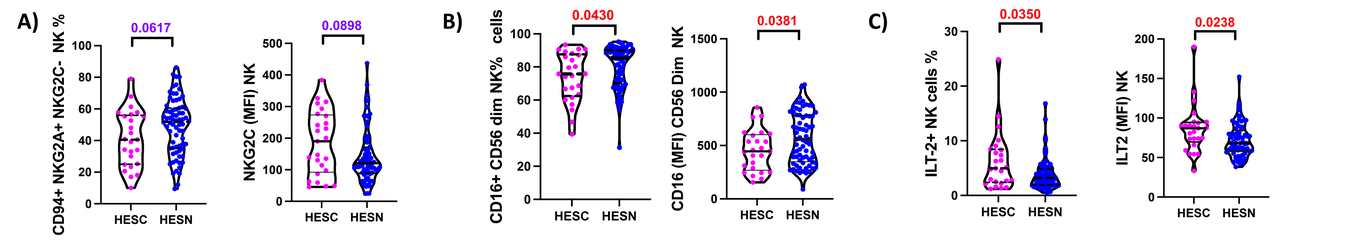


Figure 1: A) NK cells from HESC trend towards a more differentiated phenotype, with tendencies of lower expression of NKG2A and higher expression of the maturation marker NKG2C. **B)** A significantly lower expression of the ADCC marker CD16 was observed in NK cells from HESC and **C)** increased expression of the inhibitory receptor ILT-2.

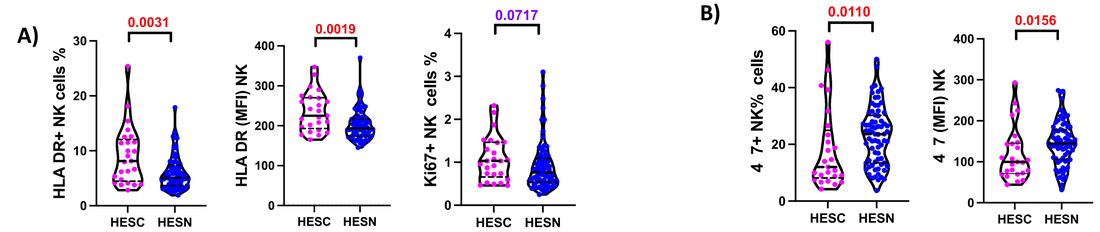
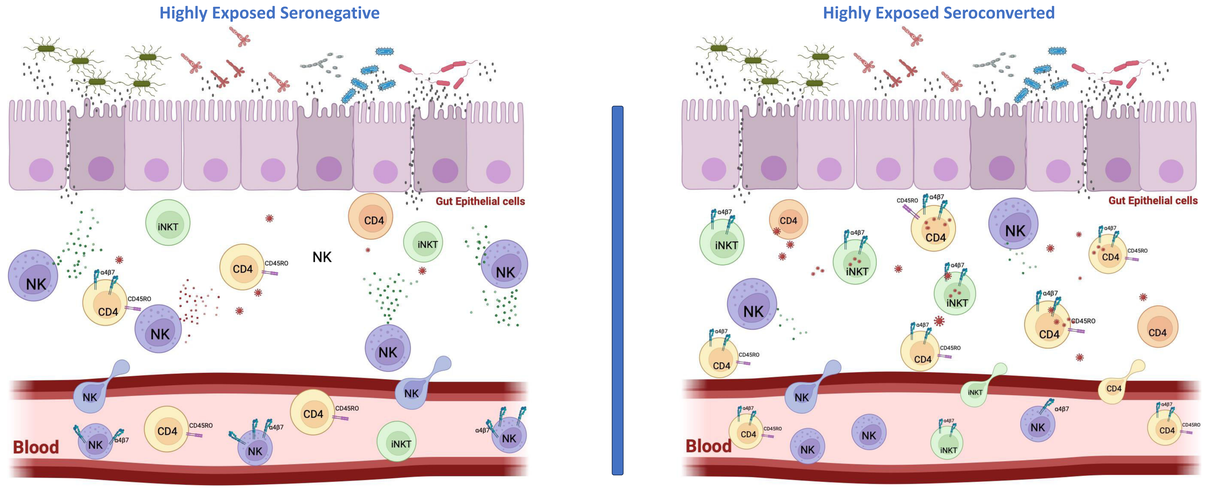


Figure 2: A-B) NK cells from HESC are more activated and have a reduced potential to migrate to the gut mucosa as shown by the levels of HLA-DR, Ki67, and $\alpha 4\beta 7$.

Conclusion

Our findings suggest that NK cells from HESC have an exhausted phenotype and poor ability to migrate to the gut mucosa where initial anti-viral responses would occur, increasing the risk of HIV acquisition.



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The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.

GLOBAL INFECTIOUS DISEASES