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In *KIR3DL1/S1* heterozygotes are KIR3DL1 and KIR3DS1 co-dominantly expressed?

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Background

Epidemiological studies show that co-carriage of *KIR3DS1* (*3DS1*) and a subset of *HLA-Bw4* alleles with an isoleucine at position 80 of the HLA heavy chain (*3DS1+*80I*) is associated with slow time to AIDS. NK cells from *3DS1+*80I* carriers have high anti-HIV activity. There is conflicting information regarding whether NK cells from *KIR3DL1/S1* heterozygotes (htz) exhibit allelic exclusion or co-dominance in terms of expression of the NK cell receptors 3DS1 and KIR3DL1 (3DL1). Monoclonal antibody (mAb) Z27 binds 3DL1 with a high and 3DS1 with a low intensity. Z27 staining cannot distinguish whether $Z27^{high}$ NK cells express 3DL1 only or include NK cells that also express 3DS1. We developed an approach to address this question.

Methods

Peripheral blood mononuclear cells from five *3DL1/S1*htz and, as controls, one *3DL1* and one *3DS1* homozygote were stained with anti-CD3, anti-CD56 and Z27. All subjects were HIV seronegative. $CD3^{-}CD56^{+}$ NK cells were sorted into $Z27^{high}$, $Z27^{dim}$ and $Z27^{neg}$ sub-populations. RNA was extracted from each subset and cDNA was prepared from RNA. Real-time (RT)-PCR was used to determine the presence or absence of *3DS1* and *3DL1* transcripts in sorted cells.

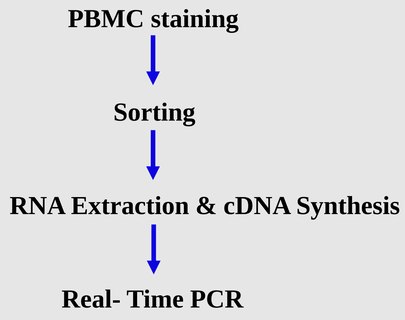

Figure 1. Sample preparation for sorting and Real-Time PCR

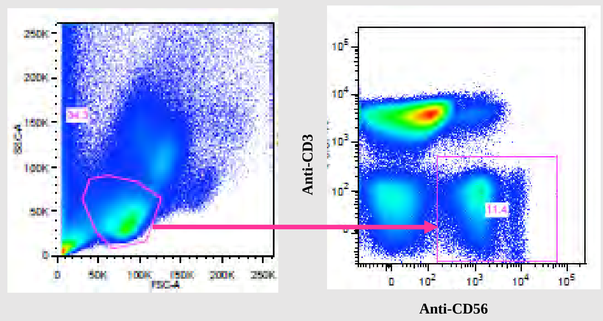

Figure 2. Strategy for gating on NK cells ($CD3^{-}$ $CD56^{+}$)

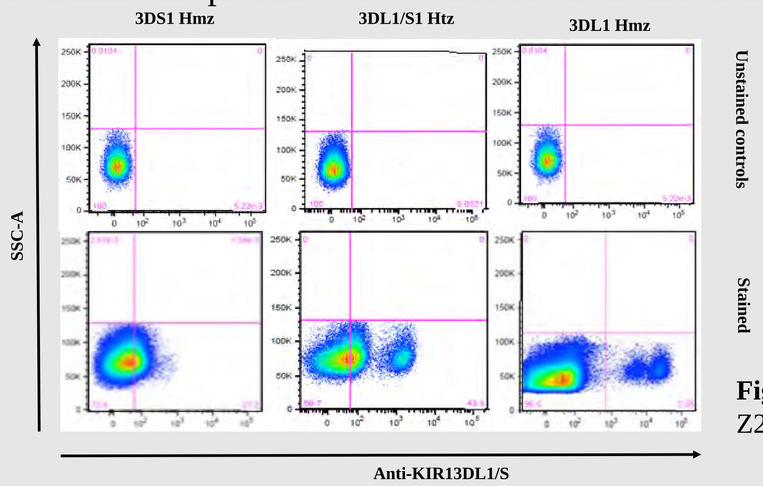

Figure 3. Gating strategy used to separate Z27high, dim and negative populations**.**

Results

First, we verified the specificity of *3DS1*, *3DL1* primer sets. We verified that the sorting gates distinguishing $Z27^{dim}$ from $Z27^{neg}$ populations were correctly set by showing that no *3DS1* transcripts were present in the $Z27^{neg}$ subset from either *3DS1*hmz or *3DL1/S1*htz. Using RT- PCR and the *3DS1* specific primers we found that the $Z27^{dim}$ subset of *3DS1*hmz and both the $Z27^{dim}$ and $Z27^{high}$ subsets of *3DL1/S1*htz amplified *3DS1* transcripts. We found *3DL1* primers amplified transcripts from $Z27^{high}$, but not from the $Z27^{dim}$ subset of *3DL1/S1*htz.

Table 1. Z27 staining subsets detected in NK cells from carriers of the generic *KIR3DL1/S1* genotypes

	Z27 dim	Z27 high
3DS1 hmz	Yes	No
3DL1/S1 htz	Yes	Yes
3DL1 hmz	No	Yes

Table 2. RT- PCR results for different subsets of sorted NK cells

Transcripts	3DS1 hmz dim	3DL1/S1 dim	3DL1/S1 high
3DL1	No	No	Yes
3DS1	Yes	Yes	Yes

Conclusion:

3DL1 and 3DS1 can be co-dominantly expressed on NK cells. The accepted theoretical paradigm posits that engagement of the inhibitory 3DL1 NK receptor by its ligand, HLA-Bw4, is important in licensing NK cells for function. Engagement of the activating 3DS1 NK receptor should tune down the potency of NK cell licensing and the functional potential of 3DS1+ NK cells. This raises questions regarding the mechanism underlying the association between *3DS1+*80I* carriage and HIV control. Our results provide a framework for improving our understanding of the role played by the 3DL1 and 3DS1 receptors in determining the function of NK cell subsets in general and the anti-HIV function of these cells in particular.

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