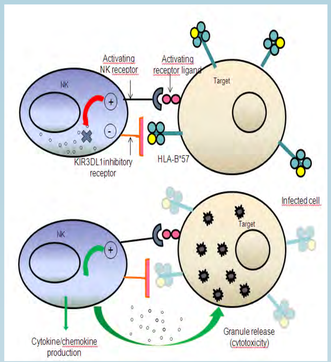
Do KIR3DL1/HLA-B Combinations Influence ADCC Responses of HIV-1 Infected Slow Progressors?

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BACKGROUND:

The *h/*y+B*57 genotype, which encodes high expression Killer Immunoglobulin-like Receptor (KIR)3DL1 variants, and HLA-B*57 is the KIR/HLA combination with the strongest effect on slowing time to AIDS and controlling HIV viral load, as compared to Bw6 homozygotes (Bw6hmz). The interaction of 3DL1 with HLA-B*57 is a potent natural killer (NK) cell licensing combination. The percentage of functional NK cells induced by HLAnull or autologous HIV-infected CD4 cells is higher when from *h/*y+B*57 carriers than from Bw6hmz or carriers of several other 3DL1hmz/HLA-Bw4 combinations. Using cells from HIV-infected slow progressors (SP) we investigated whether KIR3DL1/HLA genotypes that support licensing (3DL1+Bw4) generate NK cells with higher ADCC activity than those that do not (3DL1+Bw6hmz).



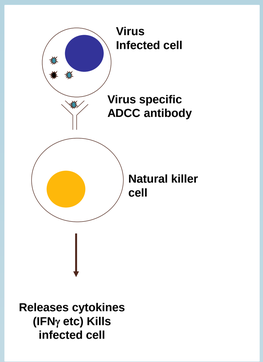


Figure 1. Mechanism of function of NK cells

Table 1: Classification of KIRs with their HLA Ligand

	Receptor	Ligand
Inhibitory	KIR2DL1	HLA-C2
	KIR2DL2	HLA-C1
	KIR2DL3	HLA-C1
	KIR3DL1	HLA-Bw4
	KIR3DL2	HLA-A3/11

*Both inhibitory & activating capabilities
?Unknown ligand

Nomenclature: KIR xDy #
x = number of Ig domains
y = Long (L) or Short (S) cytoplasmic domain
# = distinct form

KIR DLs are inhibitory and contain ITIM(s) in cytoplasmic domain
KIR DSs are activating and have charged interactions with the adaptor DAP-12

KIR3DL1 homozygotes can be further classified into 2 categories: *h/*y and *l/*x

1. *h/*y = high expression allele with no low expression allele
   i.e. *y is either *h or *004 (null)
2. *l/*x = has at least 1 low expression allele
   i.e. *x is either *h, *l, or *004.

HYPOTHESES

1. NK cells from HIV-infected SP mediate ADCC activity.
2. NK cell licensing influences the potency of ADCC activity.

METHODS

We studied 48 *3DL1hmz* SP with plasma VL <3000 cells/mm^3, including carriers of *HLA-Bw4+HLA-B*57-* (n=34), *Bw6hmz* (n=12) and **h/*y+B*57* (n=6) genotypes. Anti-HIV ADCC was measured using a GranToxiLux (GTL) assay, which assesses the ability of NK cells (effectors) to deliver granzyme B (GzB) to HIV gp120-coated CEM.NKr.CCR5 targets (%GzB+ cells) in the presence of a common pooled source of anti-HIV IgG. The significance of between-group differences was assessed using the Mann-Whitney test.

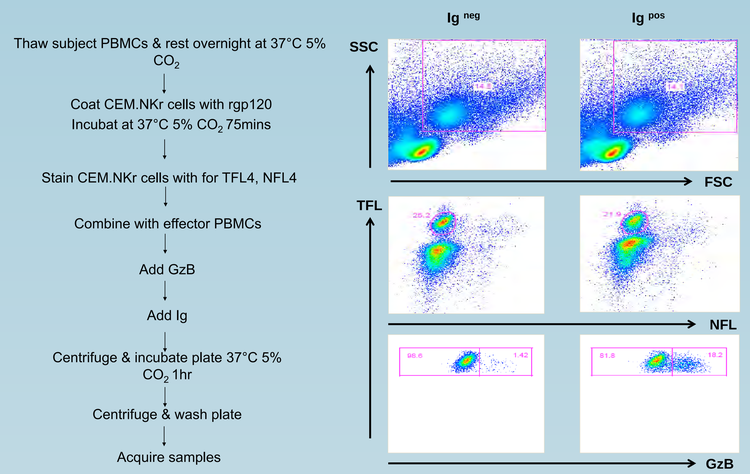


Figure 2. Method and gating strategy used to measure ADCC activity using the GranToxiLux assay.

The GTL assay combines CEM-NKr cells coated with rgp120 (targets), subject PBMCs (effectors) and a common source of pooled Ig. The rgp120 coated CEM-NKr cells were also stained with a target cell dye (TFL4), a viability dye (NFL1) and the GzB substrate, which is taken up by the target cells. Cleavage of GzB occurs as a direct result of target cell death by NK cells. Therefore, the percent killing of target cells was determined by the percentage of GzB positive target cells in the presence of Ig.

Table 2: KIR3DL1hmz Study Population *h/*y vs *l/*x and Bw4/Bw6 genotype categorisation

Subject #	Bw4/6	Bw4 at HLA-A locus	B57	KIR3DL1 genotype	HLA-A		HLA-B	
1	Bw4	0	1	*/*x	A*02:01	A*02:01	B*57:01	B*57:01
2	Bw4	0	0	*/*x	A*02:05	A*74:02	B*27:03	B*49:01
3	Bw4	0	0	*/*x	A*02:01	A*02:05	B*53:01	B*58:01
4	Bw4	0	0	*/*x	A*03:01	A*30:02	B*27:03	B*51:01
5	Bw4	0	0	*h/*y	A*02:01	A*02:01	B*44:02	B*44:02
6	Bw4	0	1	*h/*y	A*02:01	A*30:01	B*13:02	B*57:01
7	Bw4	0	1	*h/*y	A*02:02	A*30:02	B*53:01	B*57:03
8	Bw4	1	0	*h/*y	A*02:05	A*23:01	B*49:01	B*53:01
9	Bw4	0	0	*/*x	A*03:01	A*34:02	B*44:03	B*53:01
10	Bw4	0	0	*h/*y	A*03:01	A*11:01	B*51:01	B*51:01
11	Bw4	0	1	*/*x	A*01:01	A*11:01	B*13:01	B*57:01
12	Bw4	0	0	*/*x	A*01:01	A*02:01	B*15:10	B*15:10
13	Bw4	0	1	*h/*y	A*01:01	A*30:01	B*27:03	B*57:01
14	Bw4	0	0	*h/*y	A*02:01	A*26:01	B*44:02	B*52:01
15	Bw4	0	0	*004 hmz	A*02:06	A*30:01	B*13:02	B*27:05
16	Bw4	0	0	*h/*y	A*02:01	A*03:01	B*51:01	B*52:01
17	Bw4	0	1	*h/*y	A*02:01	A*30:01	B*13:02	B*57:01
18	Bw6	0	0	*/*x	A*02:01	A*03:01	B*07:02	B*18:01
19	Bw6	0	0	*/*x	A*02:01	A*30:01	B*07:02	B*42:01
20	Bw6	0	0	*/*x	A*68:02	A*74:02	B*15:10	B*41:01
21	Bw6	0	0	*h/*y	A*02:06	A*31:01	B*15:01	B*15:01
22	Bw6	0	0	*h/*y	A*02:01	A*03:01	B*07:02	B*48:01
23	Bw6	1	0	*/*x	A*02:01	A*25:01	B*18:01	B*35:01
24	Bw6	1	0	*h/*y	A*23:01	A*23:01	B*42:01	B*45:01
25	Bw6	0	0	*h/*y	A*01:01	A*03:01	B*07:02	B*14:02
26	Bw6	1	0	*h/*y	A*02:01	A*32:01	B*14:01	B*14:06
27	Bw6	0	0	*/*x	A*01:01	A*30:02	B*08:01	B*81:01
28	Bw6	0	0	*/*x	A*03:01	A*68:02	B*07:02	B*14:02
29	Bw6	0	0	*h/*y	A*02:01	A*02:05	B*41:01	B*48:01
30	Bw6	1	0	*h/*y	A*25:01	A*74:10	B*35:01	B*40:01
31	Bw6	0	0	*/*x	A*03:01	A*31:01	B*14:02	B*40:01
32	Bw6	1	0	*/*x	A*03:01	A*24:02	B*07:05	B*40:02
33	Bw6	0	0	*h/*y	A*02:01	A*11:01	B:14:02	B*40:06
34	Bw6	0	0	*h/*y	A*03:01	A*03:01	B*07:02	B*07:05
35	htz	0	0	*h/*y	A*02:01	A*02:01	B*27:05	B*40:02
36	htz	0	1	*h/*y	A*02:02	A*33:03	B*35:01	B*57:01
37	htz	0	0	*h/*y	A*01:01	A*01:01	B*08:01	B*27:05
38	htz	0	0	*h/*y	A*11:01	A*11:01	B*44:03	B*55:01
39	htz	0	1	*h/*y	A*01:01	A*68:02	B*57:03	B*73:01
40	htz	0	0	*h/*y	A*02:01	A*02:01	B*40:02	B*52:01
41	htz	0	0	*h/*y	A*02:01	A*02:01	B*15:03	B*44:02
42	htz	0	0	*h/*y	A*29:01	A*66:02	B*14:02	B*58:01
43	htz	0	0	*h/*y	A*02:01	A*02:01	B*07:02	B*51:01
44	htz	0	0	*h/*y	A*03:02	A*68:02	B*14:02	B*44:03
45	htz	1	0	*h/*y	A*03:01	A*68:02	B:14:02	B*37:01
46	htz	0	0	*/*x	A*02:01	A*68:02	B*14:02	B*44:02
47	htz	1	0	*/*x	A*24:02	A*24:03	B*18:01	B*52:01
48	htz	0	0	*h/*y	A*01:01	A*68:01	B*08:01	B*44:02

RESULTS

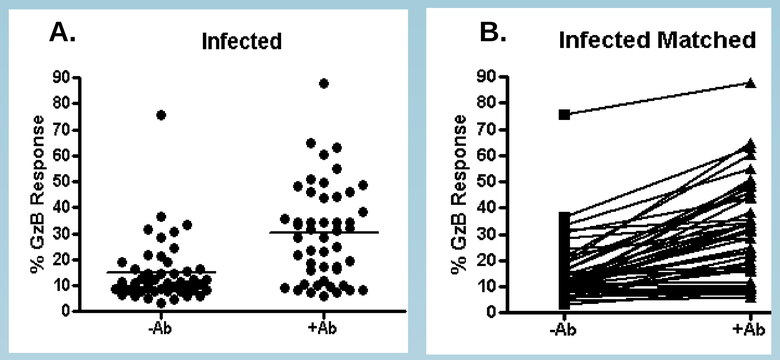


Figure 3. NK cells from HIV-infected SP subjects mediate ADCC.
A. NK cells from *3DL1hmz* mediated significantly higher levels of ADCC in the presence versus absence of pooled plasma Ig (p<0.0001). **B.** Matched pairs test showing %GTL activity in the presence versus absence of Ig in the 48 subjects tested (p<0.0001).

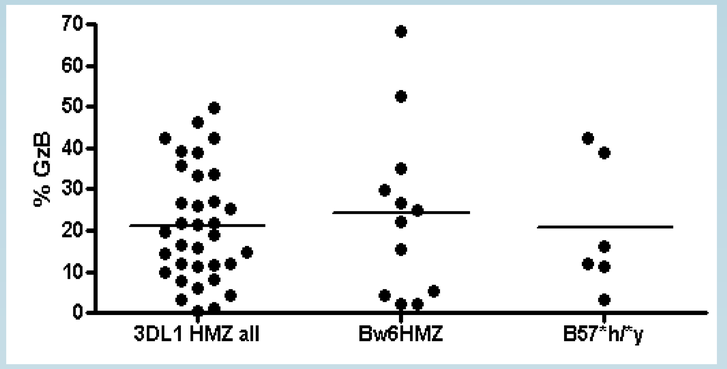


Figure 4. *HLA-Bw4+* and *Bw6hmz 3DL1hmz* individuals exhibited similar levels of ADCC.
A. NK cells from *3DL1hmz* who were *HLA-Bw4+* or *Bw6hmz* mediated equivalent levels of ADCC (21.1 ± 13.8 vs 24.05 ± 20.7 %GzB cells+, p=0.87). NK cells from **h/*y+B*57* carriers also mediated similar levels of ADCC activity (20.7 ± 16.1) as those from *Bw6hmz* (p=0.96) and carriers of other *Bw4* alleles (p=0.85).

CONCLUSIONS

- NK cells from HIV-infected SP mediate ADCC.
- Carrying KIR/HLA combinations that play a role in NK cell licensing through 3DL1 ligation does not influence the potency of ADCC activity of NK cells from HIV-infected SP.
- This analysis is being replicated in HIV seronegative subjects to assess whether HIV infection may abrogate the influence of licensing on ADCC even in this well controlled population.

ACKNOWLEDGMENTS

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Model for anti-HIV control by NK cells from subjects positive for *h/*y+B*57

