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FULL TITLE: HIV-1 Evades a Gag Mutation that Abrogates KIR Binding and Disinhibits NK Cells in Infected Individuals with KIR2DL2^{+}/HLA-C*03:04^{+} Genotype

SHORT TITLE: HIV-1 clade C avoids KIR2DL2 binding

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Abstract

HIV-1 sequence variations impact binding of inhibitory killer cell immunoglobulin-like receptors (KIRs) to human leucocyte class I (HLA-I) molecules modulating NK cell function. HIV-1 strains encoding amino acids that mediate binding of inhibitory KIRs might therefore have a selective benefit in individuals expressing the respective KIR/HLA genotypes. Here we demonstrate that HIV-1 clade C avoids a **p24** Gag mutation that abolishes binding of KIR2DL2 to HLA-C*03:04 and disinhibits NK cells in individual encoding for this genotype.

Natural killer (NK) cells are an important component of the innate immune system, able to rapidly respond to target cells without prior sensitization. The effector function of NK cells to stimuli are determined by the interplay between activating and inhibitory receptors [1], including killer immunoglobulin receptors (KIRs). KIRs bind to human leukocyte antigen class I (HLA-I) molecules to monitor HLA-I expression levels on cells, and the affinity of these KIR/HLA-I interactions is dependent on the sequence of the HLA-I presented peptide [2, 3]. KIR/HLA-I compound genotypes have been associated with the speed of HIV-1 disease progression [4], and selection of viral sequence polymorphisms associated with specific KIR or KIR/HLA-I compound genotypes in HIV-1 infection have been reported **[5-7]**, indicating KIR+ NK cell-mediated immune selection pressure.

We previously showed selection of two minor HIV-1 sequence polymorphisms in a cohort of 392 clade C HIV-1 infected South Africans and demonstrated that these mutations within p24 Gag were associated with combined expression of KIR2DL3 and HLA-C*03:04 genotypes and mediated escape from NK cell responses through enhanced engagement of inhibitory KIR2DL3 [8]. Here we investigated another sequence polymorphism at position 84 of p24 Gag ($T_{Gag84}V$) associated with the combined HLA-C*03:04/KIR2DL2 genotype in this cohort. While 42 infected individuals expressing both KIR2DL2 and HLA-C*03:04 all harbored viruses encoding for the major variant threonine (T) at amino acid position 84 of HIV-1 Gag, the minor variant valine (V) at position 84 was not present in the $KIR2DL2^{+}$/HLA-$C*03:04^{+}$ individuals yet was present in 10% (35/350) of individuals not encoding for KIR2DL2 and HLA-C*03:04 (**see section A of Supplementary Material;** Fig 1A , http://links.lww.com/QAD/B855, P=0.02, Fischer's Exact test). These data suggest a significant advantage for HIV-1 to encode for T_{Gag84} in $KIR2DL2^{+}$/HLA-$C*03:04^{+}$ individuals. The $T_{Gag84}V$ polymorphism is located within the C-terminal region of the HLA-C*03:04-restricted peptide RSLYNTVA**T**L (RS**T**L10). The major variant (RSLYNTVA**T**L (RS**T**L10)) and the minor variant (RSLYNTVA**V**L (RS**V**L10)) peptides equally stabilized HLA-C*03:04 expression **(method described in section B of Supplementary Material** , http://links.lww.com/QAD/B855**)** on transfected TAP-deficient 721.221 cells (221-TAPko-HLA-C*03:04) (T [mean 2.2 ± 0.20 SD] to V [mean 2.3± 0.26SD], P=0.8), and resulted in an up to 3-fold higher expression of HLA-C*03:04 compared to non-peptide-pulsed control cells (Fig 1B). These HLA-C*03:04-stabilization data demonstrated that the $T_{Gag84}V$ polymorphism identified within HIV-1 p24 Gag did not impact the ability of the respective peptides to bind to HLA-C*03:04.

Next, the RS**T**L10 and RS**V**L10 peptides were assessed for their ability to mediate KIR2DL2-binding to HLA-C*03:04, using a KIR2DL2-Fc fusion construct, as previously described [8], **(see also section C of Supplementary Material** , http://links.lww.com/QAD/B855**).** The HLA-C*03:04-presented GAVDPLL**A**L (GAL) peptide **derived from the human importin-α-1 subunit (residues 204-212)** [9], is known to bind KIR2DL2 [9] and was used as positive control, whereas the variant GAVDPLL**K**L (GKL) peptide which stabilizes HLA-C*03:04 at similar levels but does not bind KIR2DL2 [9] was

used as a negative control. Results from 5 independent KIR2DL2-Fc-binding assays consistently demonstrated weaker binding of KIR2DL2-Fc to HLA-C*03:04 presenting the minor variant peptide RS**V**L10, while the major variant peptide RS**T**L10 mediated significantly higher binding of KIR2DL2-Fc to HLA-C*03:04 (Fig 1C, P=0.008, Mann Whitney test). Higher KIR2DL2-Fc-binding furthermore had significant functional consequences for $KIR2DL2^+$ NK cells, as demonstrated by increased inhibition of CD107a expression as a read-out for NK cell activation [10], **(Supplementary Figure 1** , http://links.lww.com/QAD/B855**)**, of $KIR2DL2^+$ primary NK cells following co-incubation of 221-TAPko-HLA-C*03:04 cells pulsed with the major variant RS**T**L10 peptide compared to the minor variant RS**V**L10 peptide (**method described in section D of Supplementary Material** , http://links.lww.com/QAD/B855) (Fig 1D, P=0.007, Mann Whitney test, N = 8). In summary, the results from these KIR2DL2/HLA-C*03:04 binding assays and functional assays using $KIR2DL2^+$ primary NK cells demonstrate that the minor V_{Gag84} HIV-1 variant that was circulating in KIR2DL2/HLA-C*03:04 negative individuals, but not detected in individuals encoding for KIR2DL2/HLA-C*03:04, mediated significantly reduced binding of KIR2DL2 to HLA-C*03:04, resulting in stronger activation of primary $KIR2DL2^+$ NK cells.

Previous studies have reported minor HIV-1 sequence polymorphisms selected in individuals expressing specific KIRs or KIR/HLA-I combinations that enhanced binding of inhibitory KIRs and inhibit KIR^+ NK cells, consistent with KIR^+ NK cell-mediated selection of viral escape variants **[3, 6-8]**. Here we report a sequence polymorphism within HIV-1 Gag ($T_{Gag84}V$) for which the major variant (T_{Gag84}) was encoded in all individuals expressing KIR2DL2/HLA-C*03:04, while the minor variant (V_{Gag84}) was detected in some individuals not encoding for this KIR/HLA compound genotype. The combined KIR2DL2/HLA-C*03:04 genotype is frequent in the studied population of HIV-1 clade C infected individuals from South Africa (42 of 392 individuals, 10.7%), and these observations are consistent with the selection and potential accumulation of the T_{Gag84} variant over time, similar to what has been described for HLA-B*51- restricted epitope TAFTIPSI in different populations [11]. The current study was a cross sectional analysis in chronically HIV-1 clade C infected individuals in which the timing of HIV-1 infection was not known. It was therefore not possible to determine whether the T_{Gag84} polymorphism was indeed selected in $KIR2DL2^+$/HLA-$C*03:04^+$ individuals infected with the minor V_{Gag84} variant; and the fact that less than 10% of circulating viruses in these 392 individuals encoded for V_{Gag84} suggests that most individuals were initially infected with the T_{Gag84} polymorphism. Longitudinal studies with accurate timing of primary infection will be required to determine whether the T_{Gag84} variant is indeed selected in $KIR2DL2^+$/HLA-$C*03:04^+$ individuals infected with the minor V_{Gag84} variant, leading to the accumulation of the T_{Gag84} variant over time. In summary, these data suggest potential accumulation of sequence polymorphisms that reduce recognition by KIR^+ NK cells over time, eventually representing the major variant in the circulating HIV-1 strains, similar to the enrichment of HIV-1 variants selected in response to $CD8^+$T-cell-mediated immune pressure [11].

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Author contributions

MZ, MA and CT conceived and designed the experiments. MZ, KN, AN and SN performed experiments. JM, ZM, NI, MA, PG, and TN contributed reagents/materials/analysis tools and enrolled patients. MZ, KN, AN, and CT analyzed the data. MZ, MA and CT wrote the first draft of the manuscript. All authors commented on the manuscript and agree with the results and conclusions.

Conflicts of interest

The authors declare no conflicts of interest. The work was supported by the Molecular Mechanisms of Viral Pathogenesis Program of the Heinrich Pette Institute. CT was supported by the Wellcome Trust (102468/Z/13/Z). The Sinikithemba cohort was funded by the NIH (Grant ROI-AI067073 Contract NOI-AI-15422). This study was also supported in part through the South African Research Chairs Initiative, International AIDS Vaccine Initiative (IAVI) (grant # UKZNRSA1001) and the Victor Daitz Foundation. The views expressed in this publication are those of the authors and not of the funders.

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Figure Legend

Figure 1. Reduced KIR2DL2-binding and enhanced KIR2DL2$^+$ NK cell activity in response to the minor V_{Gag84} HIV-1 variant. A) Distribution of major T_{Gag84} and minor V_{Gag84} variant Gag sequences in viruses from individuals not encoding for both KIR2DL2 and HLA-C*03:04 (n=350) and KIR2DL2$^+$/HLA-C*03:04$^+$ individuals (n=42), P=0.02 calculated using Fischer's Exact test. B) Equal stabilization of HLA-C*03:04 expression on 221-TAPko-HLA-C*03:04 cells with the RSLYNTVA**T**L peptide (RSTL10) and the RSLYNTVA**V**L peptide (RSVL10) containing the $T_{Gag84}V$ sequence polymorphism. The HLA-C*03:04-binding peptides GAVDPLLAL (GAL) and GAVDPLLKL (GKL) were used as controls. HLA-C*03:04 expression is indicated by relative fluorescence intensity normalized to no peptide control. Data represents mean of 3 experiments with error bars indicating SD. C) Binding of KIR2DL2-Fc to 221-TAPko-HLA-C*03:04 cells pulsed with the GAVDPLLAL (GAL), GAVDPLLKL (GKL), RSLYNTVA**T**L (RSTL10) or RSLYNTVA**V**L (RSVL10) peptides. Results from five independent experiments are shown, lines represent median. P values were calculated using Mann Whitney test. D) Relative inhibition of degranulation (quantified by CD107a expression) of primary KIR2DL2$^+$ NK cells by 221-TAPko-HLA-C*03:04 cells pulsed with the GAVDPLLAL (GAL), GAVDPLLKL (GKL), RSLYNTVA**T**L (RSTL10) or RSLYNTVA**V**L (RSVL10) peptides. CD107a expression of NK cells in response to HLA-I deficient 221 cells was set at 100%, and relative inhibition of KIR2DL2$^+$ NK cell in response to target cells presenting the different peptides was calculated. Primary NK cells from eight healthy KIR2DL2$^+$ participants were tested, lines represent median. P values were calculated using Mann Whitney test.