HIV Cell Tropism Dependent Impact of CTL Escape Mutations in Gag on Viral Replicative Capacity

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Introduction

KK10 (K_{263}-K_{272}) within the N-terminus of HIV p24/Gag represents the immunodominantly targeted epitope in the context of HLA-B*27$^+$ HIV-infected individuals. Due to its association with delayed disease progression B*27 is considered to be a protective allele during HIV infection. The major escape mutation $R_{264}K$ alters the epitope in a way that it is no longer presented by B*27, causing a substantial reduction in viral replicative capacity at the same time. This defect can be partially attributed to an altered interaction between p24 and the cellular protein Cyclophilin A (Schneidewind et al., 2007). To our knowledge, all work regarding the compromised replication phenotype of $R_{264}K$, including its stereotypic reconstitution *in vivo* by $S_{173}A$, was done utilizing CXCR4 (X4) tropic HIV-1 strains (e.g. NL4-3). Since the R5/X4 tropism shift usually occurs rather late in the course of HIV infection and CCR5$^+$ (R5) cells are a major reservoir for HIV it would be important to confirm these findings with R5-tropic viruses (e.g. YU2; see methods) and their respective target cells. We analyzed two viral variants with HLA-B*27 associated mutations, the double escape mutant $R_{264}K$, $L_{268}M$ (RKLM) and the fully compensated variant containing $S_{173}A$, $R_{264}K$ and $L_{268}M$ (SARKLM). Additionally, we analyzed the replicative capability of the viral variant QVINA ($H_{219}Q$, $I_{223}V$, $M_{228}I$, $T_{242}N$ and $G_{248}A$). This set of mutations is associated with successful escape of the immunodominant HLA-B*57 restricted TW10 epitope (T_{241}-W_{250} within the N-terminus of HIV p24/Gag) (Brockman et al., 2007).

Results

To test our hypothesis that particular mutations in p24, associated *in vivo* with CTL escape, differentially impact replication capacity in X4$^+$ and R5$^+$ host cell populations, we infected in a first approach X4$^+$, R5$^+$ double positive GXR25 cells with the generated viral variants containing the KK10-associated epitope-variants (Fig. 1). The ratios between the chimeric R5-tropic variants and the chimeric WT match essentially the ratios of the respective X4-tropic NL4-3 viruses. This lack of variation indicates that the different viral 3'halfes do not impact the relative replication capacities of the p24 variant viruses in GXR25 cells.

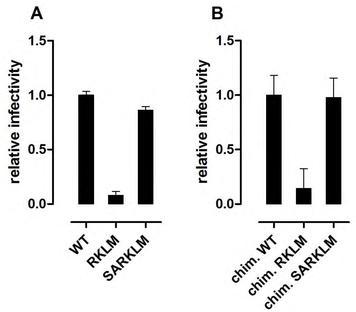


Fig.1: Relative percentage of infected GXR25 cells after infection with the X4-tropic NL4-3 variants (A) and the chimeric R5-tropic variants (B) is shown.

Results

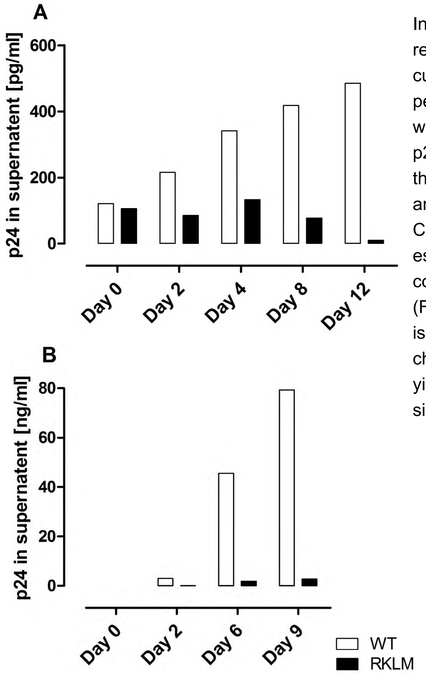


Fig.2: Kinetics of the accumulation of p24 of WT (white bars) and RKLM (black bars) in PBMCs (A) and MDMs (B) is shown over period of several days.

In a second approach we tested the viral variants in primary cells with the respective co-receptor. To asses the replication capacities of the different viral variants the collected cell culture supernatants of monocyte derived macrophages (MDMs) and monocyte depleted peripheral blood mononuclear cells ($PBMCs_{-M}$) of three different donors were inoculated with the respective viruses. Supernatants were monitored over a period of several days for p24 content using a p24-ELISA (Fig. 2). With respect to the kinetic of p24 concentration in the supernatants of the WT (white bars) and RKLM (black bars) viruses, $PBMC_{-M}$ were analyzed at day 8 and MDMs on day 9 for the data shown in Fig. 3.

Contrary to the findings of viral replication in the GXR25 cell line, the fully compensated escape variants SARKLM and QVINA display reduced p24 amounts in MDMs when compared to the relative p24 amounts measured in the supernatants of infected $PBMCs_{-M}$ (Fig. 3, white and black bares, respectively). In MDMs, p24 yield of the chimeric SARKLM is significantly reduced compared to chimeric WT in two of the three donors, while the chimeric QVINA shows a trend towards lower yield in all three donors. In contrast, p24 yield of SARKLM is similar to that of WT when $PBMC_{-M}$ are infected and QVINA produces significantly (twofold) more p24 than the WT in all three donors (white bars).

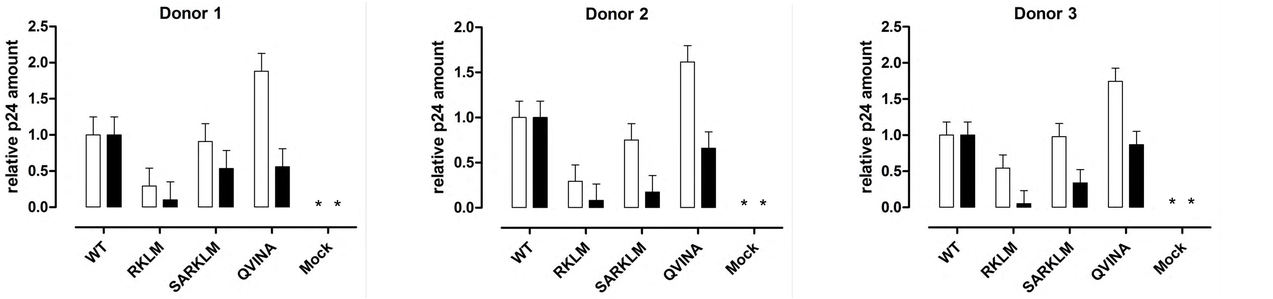


Fig.3: NL4-3 variants were used to infect $PBMC_{-M}$ (white bars) and the respective chimeric NL4-3/YU2 variants were used to infect MDMs (black bars). Asterisks indicate no measurable p24 amounts.

Methods

Cells: Monocyte derived macrophages (MDMs) were enriched from whole peripheral blood mononuclear cells (PBMCs) by negative selection (Milteny, Monocyte Isolation Kit II). Purity of the monocyte fraction was assessed by flowcytometry of CD14 (BD) labeled cells and found to be between 92 and 97%.

Infection assays: Infection of primary cells were performed in triplicates with cells from three male donors. Viral replication was monitored over a period of several days by measuring p24 content in supernatant via a p24 specific ELISA (Alliance). Infections of GXR25 (a CXCR4$^+$ and CCR5$^+$ CEM cell line derivate with Tat-inducible GFP expression) cells were performed in triplicates. After 48h Tat-dependent GFP expression was assessed by flowcytometry.

Viruses: Viral variants were constructed by site directed mutagenesis of the 5'-half of the HIV lab strain NL4-3. A R5-tropic full-length virus was generated by cloning the respective mutated NL4-3 5'-half in a plasmid containing the 3'-half of the R5-tropic HIV lab strain YU2. These chimeric NL4-3/YU2 viruses were used to infect MDMs. The $PBMCs_{-M}$ were inoculated with X4-tropic NL4-3 viruses corresponding to the respective chimeric NL4-3/YU2 viruses.

Statistical Analysis: p24 amounts measured were normalized to the wild type virus, NL4-3 or chimeric NL4-3/YU2, respectively. Statistics were done by performing an univariate variance analysis with SPSS (IBM) of the normalized values. The error bars indicate a confidence interval of 95%. Differences are considered significant at the level of 0.05.

Discussion

Here we demonstrate a differential impact of specific point mutations in Gag on p24 production in the two major target cell populations, $PBMCs_{-M}$ and MDMs. The mutations encoded by these X4- or R5-tropic viruses are involved in the escape of two important HLA-B*27 / -B*57 restricted epitopes (KK10 and TW10, respectively) (Schneidewind et al., 2007; Brockman et al, 2007 and Altfeld et al., 2006). Most interestingly, while evolution of the $S_{173}A$ compensatory mutation seems to facilitate viral replication of a viral variant containing the dominant $R_{264}K$ escape mutation within the KK10 epitope in $PBMCs_{-M}$ (and in a GXR25 cell line), replication of the compensated variant still seems to be significantly compromised in MDMs of the majority of the donors. Whether this phenomenon *ex vivo* reflects the clinical observation, that HLA-B*27 dominantly exerts its protective effect during early stages of the course of the HIV infection (while viral replication then is mainly limited to the CCR5$^+$ reservoir) remains to be determined in further analyses.

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