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

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Introduction

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 GXR2 cells with the generated viral variants containing the KKO1-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'halos do not impact the relative replication capacities of the p24 variant viruses in GXR2 cells.

Fig. 1: Relative percentage of infected GXR2 cells after infection with the X4-tropic NL4-3 variants (A) and the chimeric R5-tropic variant (B) is shown.

Results

In a second approach we tested the viral variants in primary cells with the respective co-receptor. To assess the replication capacities of the different viral variant the collected cell culture supernatants of monocyte derived macrophages (MDMs) and monocytes depleted peripheral blood mononuclear cells (PBMCs) 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 RKLK (black bars) viruses, PBMCs were analyzed day 3 and MDMs on day 9 for the data shown in Fig. 3. Contrary to the findings of viral replication in the GXR2S cell line, the fully compensated escape variants SARKLM and QVINA display reduced p24 amounts in MDMs when compared to the respective p24 amounts measured in the supernatants of infected PBMCs (Fig. 3, white and black bars, 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 PBMCs are infected and QVINA produces significantly (twofold) more p24 than the WT in all three donors (white bars).

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

Discussion

Here we demonstrate a differential impact of specific point mutations in Gag on p24 production in the two major target cell populations, PBMCs 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 (KKO1 and TW10, respectively) (Schneidewind et al., 2007; Strockman et al., 2007 and Allfled et al., 2006). Most interestingly the compensatory mutation seems to facilitate viral replication of a viral variant containing the dominant R5 escape virus within the KK01 epitope (in a GXR2S 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.

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 flow cytometry of CD14 (BD)-labeled cells and found to be between 92 and 97%.

Infection assays: Infected 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 supernant via a p24 specific ELISA (Altemis). Infectious of GXR2S (a CCR4X and CCR5- CEM cell line derived with Tat- and I-tide GPP expression) cells were performed in triplicates. After 48 hr Tat-dependent GPP expression was assessed by flowcytometry.

Virus: Viral variants were constructed by site directed mutagenesis of the 5'-half of the X4-tropic NL4-3. A R5-tropic full length virus was generated by during the respective mutant 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 were infected 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 level of 0.05.