HLA-B27 Restricted CTL-Escape Mutations Increase Sensitivity of HIV-1 to Interferon α

Philipp Schommers[1,2,3], Christopher Ford[1], Angelique Hölzemer [1,3,4], Gerd Fätkenheuer[2,3] Marcus Altfeld[1,3]

[1]Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany, [2]Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany

[3] German Center for Infection Research (DZIF), Germany. [4] 1. Medizinische Klinik und Poliklinik, Universitätsklinikum Hamburg-Eppendorf

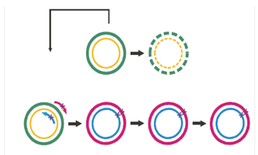
Introduction and Aims

- HLA-B27+ "Elite Controllers" have been suggested to control HIV-1 through specific cytotoxic T-cells (CTL), which exert immune selection pressure on HIV resulting in CTL-escape mutations [1].
- Better control of viremia in these patients is associated with mutations in p24 capsid [2].
- Type1 interferons (IFNs), including IFNα, upregulate cellular innate sensors with some acting as host restriction factors in CD4⁺ T cells to suppress HIV-1 replication.
- HIV-1 strains can differ in their sensitivity to IFNα-mediated restriction [3].
- Cyclophillin A (CypA) binds to intracellular HIV with opposing effects. It i) facilitates infection but also ii) acts as a intracellular sensor and cofactor for restriction factors [4].

Our aim was to determine whether the HLA-B27-restricted CTL escape mutation R264K (RK) in p24 Gag and the compensatory mutations R264K+L268M (RK+LM) and S173A+L268M+R264K (SA+RK+LM) influence the sensitivity of HIV-1 to IFNα-induced restriction.

Methods (1)

Mutagenesis

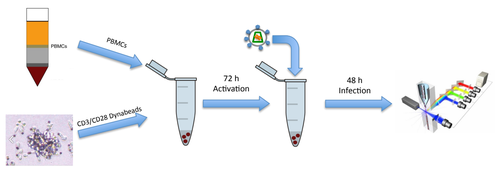


Mutations (in p24) used:

R264K (RK)	First hit mutation as seen in B27+ patients
L268M (LM)	Compensatory mutation (observed earlier than SA during the disease)
S173A (SA)	Compensatory mutation (seen at later stages of HIV-infection in patients
G221V (GV)	Mutation at CyclophilinA binding site of the capsid

Introducing the respective p24 gag mutations as seen in HLA B27+ patients into an HIV-GFP plasmid via site directed mutagenesis.

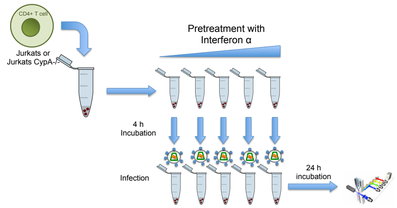
Viral fitness



Assessing the viral fitness of the respective mutated HI-viruses in Jurkat T-cells and human primary PBMCs using a FACS based technique. Cells are challenged with equal amounts of HIV-GFP (with the respective mutations). After 48h, infection rates are determined via FACS. CypA dependency of strains was tested by using "wildtype" Jurkats or Jurkat CypA -/- and human PBMCs with or without Cyclosporin A (CsA, that binds and thereby inactivates CypA).

Methods (2)

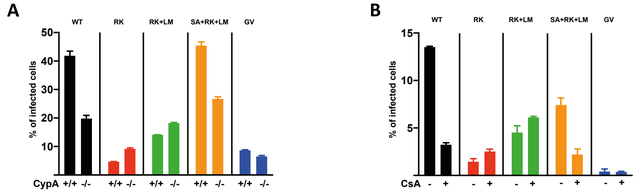
Interferon sensitivity



Assessing interferon sensitivity in Jurkats and Jurkat CypA -/-. Cells get pretreated with increasing amounts of Interferon α. After 4h cells get challenged with equal amounts of HIV-GFP (with the respective mutations, normalized via p24-ELISA). After 24h, infection rates as well as activation (CD38 expression) are determined via FACS.

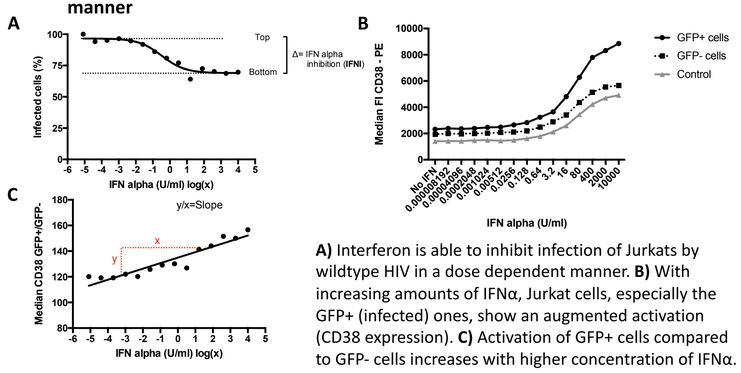
Results (1)

Figure 1: Viral fitness is highly affected by p24 escape mutations that render viruses to be CypA independent



Infection rates of the wildtype HIV-1 (WT) and the respective mutations as seen in HLA B27+ patients in **A)** Jurkat and Jurkat CypA -/- cells and in **B)** human PBMCs with or without CsA (Mean+/-SD, n=3). CTL escape mutations (RK and RK+LM) have a huge impact on viral fitness, which gets ameliorated by compensatory mutations (SA+RK+LM). HIV-1 harboring escape mutations is less CypA dependent in Jurkats as well as in human PBMCs

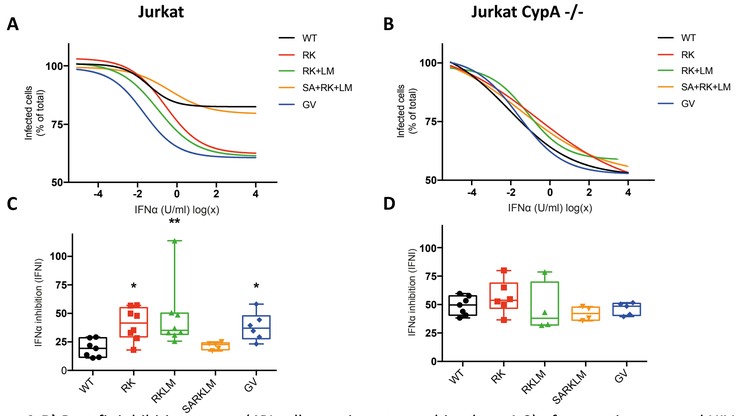
Figure 2: IFNα inhibits infection and increases activation of Jurkats in a dose depended manner



A) Interferon is able to inhibit infection of Jurkats by wildtype HIV in a dose dependent manner. **B)** With increasing amounts of IFNα, Jurkat cells, especially the GFP+ (infected) ones, show an augmented activation (CD38 expression). **C)** Activation of GFP+ cells compared to GFP- cells increases with higher concentration of IFNα.

Results (2)

Figure 3: CTL escape mutations render HIV to be more sensitive to INFα mediated restriction



A,B) Best-fit inhibition curves (4PL, all experiments combined, n= 4-8) of respective mutated HIV-1 vs WT in Jurkats and Jurkat CypA -/-. **C,D)**Calculated IFNI (see Fig. 2A) of each individual experiment. *: p=<0.05; ** p=<0.001 vs WT.

In IFNα-stimulated Jurkat cells, WT infection rates were reduced by 19%, while WT infection rates were reduced by 50% in IFNα-stimulated Jurkat CypA -/-, indicating that CypA/capsid interactions enable WT virus to evade IFNα-induced restriction factors. In contrast, RK and RK+LM were strongly inhibited by IFNα-exposure (RK: 41%; RK+LM: 35%) in Jurkats. Addition of the compensatory SA mutation (SA+RK+LM) reconstituted IFNα sensitivity to levels similar as WT, including increased susceptibility to IFNα-mediated inhibition in Jurkats CypA -/- cells.

Conclusion

- Our data suggest that HIV-1 containing the RK and RK+LM mutation are less dependent on CypA than WT.
- However, weak CypA/capsid interactions might expose these mutated strains to IFNα-induced restriction factors that compete with CypA for their binding site.
- This renders these strains more sensitive to IFNα in Jurkats.

This work highlights the role of CypA/capsid interactions in protecting HIV-1 from antiviral restriction factors, and provides a mechanism by which HLA-B27-restricted CTL escape mutations in capsid can result in a loss of viral fitness.

References & Acknowledgements

[1] **Goulder PJ, Brander C, Tang Y et al.** 2001. Evolution and transmission of stable CTL escape mutations in HIV infection. Nature **412:**334-338. [2] **Leslie AJ, Pfafferott KJ, Chetty P et al.** 2004. HIV evolution: CTL escape mutation and reversion after transmission. Nat Med **10:**282-289. [3] **Bulli L, Apolonia L, Kutzner J et al.** 2016. Complex Interplay between HIV-1 Capsid and MX2-Independent Alpha Interferon-Induced Antiviral Factors. J Virol. 2016 Jul 27;90(16):7469-80
[4] **Lahaye X, Satoh T, Gentili M et al.** 2016. Nuclear Envelope Protein SUN2 Promotes Cyclophilin-A-Dependent Steps of HIV Replication. Cell Rep. 2016 Apr 13. S2211-1247(16)30363-1

The Heinrich Pette Institute, Leibniz Institute for Experimental Virology is supported by the *Free and Hanseatic City of Hamburg* and the *Federal Ministry of Health*. Further we would like to thank the German Center for Infection Research (DZIF) that supported PS via a *Clinical Leave Stipend*.

contact: **philipp.schommers@uk-koeln.de**