Poster 197

Consequences of p24 Gag Mutations for Capsid Stability and Viral Fitness

Schommers P [1,2,4], Martrus G [1], Matschl U. [1], Hope TJ [3], Fätkenheuer G [2,4], Altfeld M.[1]

[1] Department of Virus Immunology, Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany, [2] Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany, [3] Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, United States of America [4] German Centre for Infection Research (DZIF), Cologne, Germany, [4]

contact: philipp.schommers@uk-koeln.de

Key Messages

- Cycotoxic T-lymphocytes (CTL)-driven escape mutations within p24 Gag restricted by protective HLA class I alleles have a significant impact on capsid stability.
- HLA-B57- and HLA-B27-associated CTL escape mutations T242N and R264K resulted in delayed capsid uncoating.
- Compensatory mutations observed in R264K viruses reconstituted capsid uncoating half time.
- Capsid stability was tightly correlated to kinetics of viral protein production.

Introduction

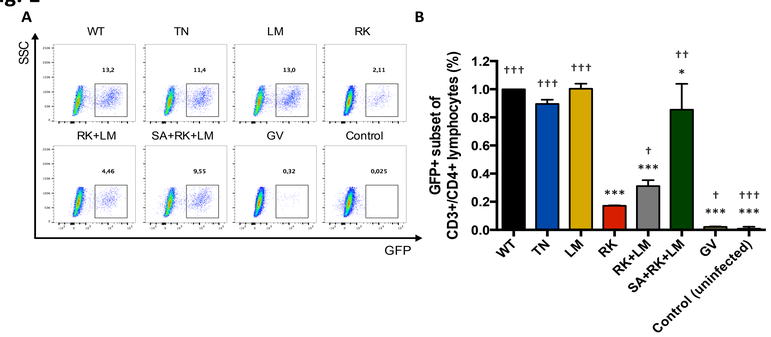
- HIV-1-infected individuals encoding for protective HLA class I alleles exhibit better control of viremia and slower disease progression [1,2].
- This has been associated with strong and highly potent HIV-1-specific CTL responses restricted by these HLA class I molecules, forcing the virus to evade immune pressure through the selection of CTL-escape mutations [3,4].
- Several of the HLA-B27 and HLA-B57-associated mutations are located in conserved areas of p24 Gag that have been suggested to be critical for capsid structure [2, 4], and *in vitro* experiments have shown that some mutations can reduce viral replication capacity [5,6].
- In this study the consequences of well-described CTL-associated p24 Gag sequence mutations for HIV-1 capsid stability were assessed using a Cyclosporine A (CsA) washout assay.

Results

- The HLA-B27-associated R264K mutation and the G221V mutation at the catalytic center of the CypA-binding site have the strongest impact on viral entry steps (Fig 1).
- Compensatory mutations of R264K in HIV-1 p24 Gag were able to restore GFP-expression in infected CD4+ T cells (Fig 1).
- The frequently occurring HLA-B57- and HLA-B27-associated CTL escape mutations T242N and R264K resulted in delayed capsid uncoating, suggesting modulation of capsid stability (Fig. 2+3).
- The described compensatory mutations L268M and S173A observed in R264K viruses reconstituted capsid uncoating half time (Fig. 2+3).
- Interestingly, capsid stability was tightly correlated to kinetics of viral protein production (Fig. 1+4).

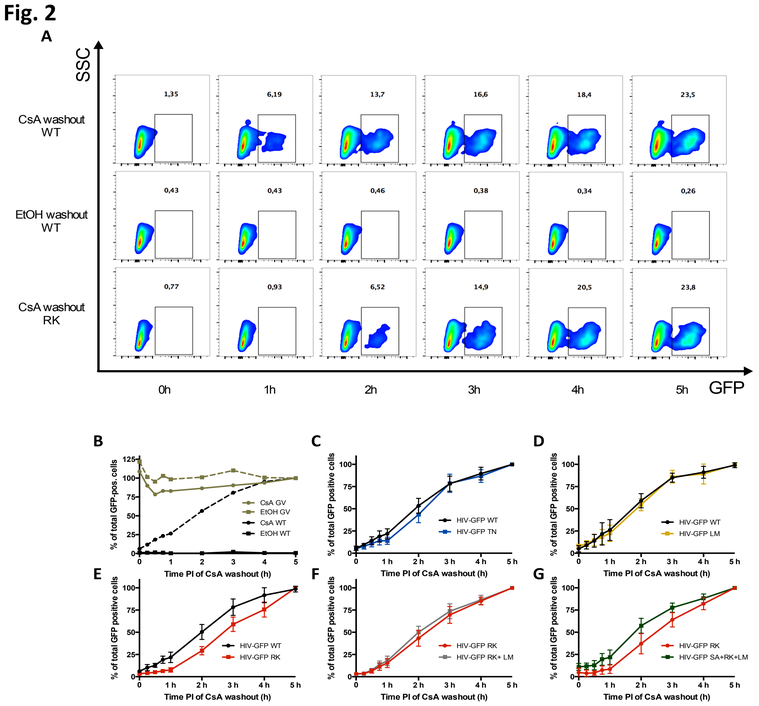
Figures

Fig. 1



Assessment of virus encoded protein production of escape mutations (TN, RK) and compensatory mutations of RK (LM, RK+LM and SA +RK+LM) in p24 Gag as selected for in HLA-B27+ individuals.
PBMCs were infected with a standardized amount of p24 of respective HIV-GFP
(A) Dot blots show GFP expression of CD3+/CD4+/CD8- lymphocytes. GFP positivity of cells indicates infected cells. Numbers show percentage of GFP+/infected cells **(B)** Fold change of infected cells over wild type (WT) that was set to 1 (n=5). *** : p=<0.0001 vs. WT; *: p=<0.05 vs. WT; ††† : p=<0.0001 vs. RK; ††:p=<0.01 †: p=<0.05 vs. RK

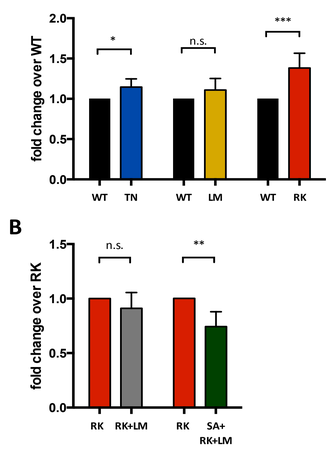
Fig. 2



GFP+ OMK cells following infection with viruses containing escape mutations and compensatory mutations after CsA washout in a time depended manner
(A) Dot blots show original data from CsA washout assay of WT and RK and EtOH washout (serves as control) of WT **(B)**CsA and EtOH washout assay of HIV-GFP WT and HIV-GFP G221V. **(C-E)** CsA washout assays comparing mutations T242N (TN, n=9), L268M (LM, n=8) and R264K (RK, n=9) to HIV-GFP WT, respectively **(F+G).** CsA washout assays comparing compensatory mutations R264K +L268M (RK+LM) and S173A+R264K+L268M (SA+RK+LM) to HIV-GFP R264K (RK), respectively (n=6).

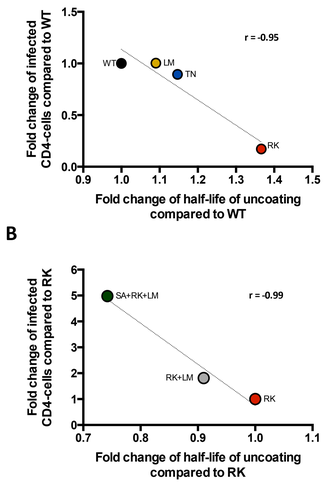
Figures

Fig. 3



Half life of uncoating of viruses containing escape mutations and compensatory mutations as calculated from the CsA washout assay
(A) WT was set to 1 and the respective escape mutations were calculated as fold change over WT. **(B)** RK was set to 1 and the compensatory escape mutations were calculated as fold change over RK.

Fig. 4



Correlation of capsid uncoating half time and viral GFP expression in CD4+ T cells
Uncoating half time and viral fitness was blotted against each other and showed a strong and significant correlation. **(A)** The fold-change of escape mutations over WT(Pearson r= -0.9522, p= 0.0478) **(B)** The fold-change of the compensatory mutations over RK (Pearson r= -0.9878, p=0.0994)

Conclusion

- Modulated capsid stability is a major determinant of the reduced percentage of GFP+ CD4+ T cells observed following infection of PBMCs with HIV-1 variants containing frequent HLA-B27- and HLA-B57-associated sequence mutations within HIV-1 capsid.
- CTL-driven escape mutations within p24 Gag restricted by protective HLA class I alleles have a significant impact on capsid stability that might contribute to the persistent control of viral replication observed despite viral escape from CTL responses .

References

[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] **Streeck H, Lichterfeld M, Alter G et al** 2007. Recognition of a defined region within p24 gag by CD8+ T cells during primary human immunodeficiency virus type 1 infection in individuals expressing protective HLA class I alleles. J Virol **81:**7725-7731.
[4] **Martínez-Picado J, Prado JG, Fry EE et al.** 2006. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. J Virol **80:**3617-3623.
[5] **Brockman MA, Schneidewind A, Lahaie M et al.** 2007. Escape and compensation from early HLA-B57-mediated cytotoxic T-lymphocyte pressure on human immunodeficiency virus type 1 Gag alter capsid interactions with cyclophilin A. J Virol **81:**12608-12618.
[6] **Schneidewind A, Brockman MA, Yang R et al** 2007. Escape from the dominant HLA-B27-restricted cytotoxic T-lymphocyte response in Gag is associated with a dramatic reduction in human immunodeficiency virus type 1 replication. J Virol **81:**12382-12393.

Acknowledgements

This work was funded by the "Deutsches Zentrum für Infektionsforschung" (DZIF) through a clinical-leave fellowship to PS and by the Heinrich-Pette-Institute, program area "Molecular mechanisms of viral pathogenesis" (MA).