Non-integrating HIV-1 is Sensitive to Inhibition by Target Cell APOBEC3A

Carsten Münk, Ananda Ayyappan Jaguva Vasudevan, Aikaterini Krikoni, Wolfgang Goering, André Franken, Daniela Marino, Gerald G. Schumann, Renate König, Wolfgang A. Schulz, Dieter Häussinger
Clinic for Gastroenterology, Hepatology and Infectiology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany

BACKGROUND
The APOBEC3A (A3A) polynucleotide cytidine deaminase of the human APOBEC3 (A3) protein family was shown to be antiviral active against HTLV-1 but not HIV-1 when expressed in the virus producer cell. In primary monocytes/macrophages, high levels of endogenous A3A activity have been associated with restriction of HIV-1 during infection. So far, ectopic expression of A3A has not been investigated to study the antiviral activity of target cell A3A.

RESULTS AND CONCLUSION
We confirm that HIV-1 is sensitive to A3A expression in the virus producer cells, due to the lack of encapsidation of A3A in viral particles. We hypothesized that HIV-1 may evade the post-entry restriction by target cell A3As by a different timing of integration into the host’s genomic DNA. Indeed, when comparing wild type with integrase mutated HIV-1, we observed that the integration-deficient virus was significantly restricted by target cell A3A. Additionally experiments in target cells showed that not only A3A, but also A3B, A3C, A3G, and A3H, but not A3D and A3F, restricted non-integrating HIV-1 post-entry. These data demonstrate that HIV-1’s sensitivity to target cell-expressed APOBEC3 proteins is modulated by the integration competence of the virus.

ACKNOWLEDGEMENTS
We thank all the members of the Münk lab for fruitful discussions. Authors gratefully acknowledge Heinz Ariansen Foundation for AIDS research, RWTH graduate school BiSoC, DFG and Jürgen Mannhiel Foundation, Molecules of Infection Graduate School for support.

APOBEC3A edits plasmid DNA, incorporate efficiently into MLV by interacting with capsid protein, but not into HIV-1 particles

Restriction of HIV-1 integration deficient virus (D64V mutant) by target cell APOBEC3

Doxycycline induced target cell A3A inhibits integration deficient HIV-1 and MLV but not wild type HIV-1 effectively

Stable target cell A3A restrict MLV and HIV-1 replication.

A3A-mediated inhibition of plasmid expression:
(a) HEK293 or b) HEK293T cells (2 x 10^6 cells/well in a 12-well plate) were co-transfected with the luciferase expression plasmid PM71-luc (200 ng), and increasing amounts of the A3A or A3A E729A expression plasmids (0.1, 0.25, 0.5, 0.75 and 1 μg) and pCΔNA1 to make up equivalent DNA concentrations for each transfection. Two days post-transfection, luciferase activity was measured to compare the expression of luciferase in different samples.
(b) A representation of plasmid editing mediated by A3A in both strands.

Results and conclusion:
We confirm that HIV-1 is sensitive to A3A expressed in the virus producer cells, due to the lack of encapsidation of A3A in viral particles. We hypothesized that HIV-1 may evade the post-entry restriction by target cell A3As by a different timing of integration into the host’s genomic DNA. Indeed, when comparing wild type with integrase mutated HIV-1, we observed that the integration-deficient virus was significantly restricted by target cell A3A. Additionally experiments in target cells showed that not only A3A, but also A3B, A3C, A3G, and A3H, but not A3D and A3F, restricted non-integrating HIV-1 post-entry. These data demonstrate that HIV-1’s sensitivity to target cell-expressed APOBEC3 proteins is modulated by the integration competence of the virus.

Acknowledgements:
We thank all the members of the Münk lab for fruitful discussions. Authors gratefully acknowledge Heinz Ariansen Foundation for AIDS research, RWTH graduate school BiSoC, DFG and Jürgen Mannhiel Foundation, Molecules of Infection Graduate School for support.