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Evaluation of HIV-1 clones for unique properties associated with transmission.

K.Klein[1], A. Ratcliff[2], G. Nickel[2], I. Nankya[2], M. Lobritz[2], Y. Gao[2], R. Shattock[3], E. Arts[1]

[1]University of Western Ontario, Microbiology and Immunology, London, Canada, [2]Case Western Reserve University, Medicine, Cleveland, United States, [3]Imperial College, Medicine, London, United Kingdom.

Background

Sexual HIV-1 infection requires penetration of the virus across the mucosal barrier and the establishment of infection in target cells. In the vast majority of cases, mucosal HIV-1 infection is initiated by a single infectious virus or a small number of HIV-1 virions. Next to CCR5 receptor usage and envelope glycosylation, little is known about the phenotypic properties of these transmitted HIV-1 virions and the influence distinct genotypic features play in the genetic bottleneck selection process.

Therefore we evaluated a number of acute/early and chronic HIV-1 viruses, isolated from the female genital tract and blood, in a series of genotypic and phenotypic assays to determine possible differences that may influence transmission fitness.

Methods

Chimeric viruses: Acute and chronic subtype B Env-chimeras were constructed using a yeast-based recombination repair system as previously described by Marozsan and Arts. PCR amplified env products were recombined into a pRECnfl$_{NL4\text{-}3\Delta}$env/URA3 vector by yeast transfection. DNA was obtained from yeast grown on FOA plates and bacterial transformation to amplify DNA for purification. Vectors were used to transfect 293T cells together with a complementing pCMV_cplt vector. Virus from transfections were propagated in U87-CD4-CCR5 cells to produce replication competent chimeric viruses..

Sensitivity to entry inhibitors: Affinofiles were incubated with serial dilutions of TAK-779, Enfuvirtide or Maraviroc for 1 hour before the addition of virus. After 2 days the IC_{50} values were determined using a luciferase reporter assay.

Entry kinetics: U87 cells were spinoculated with virus at 4° C. Virus infections were synchronised with warm media. Subsequently Nevirapin (VP) was added at various time points. Cells were incubated for 48 hours before lyses and determination of luciferase activity.

Replication efficiency: Serial dilutions of acute and chronic viruses were added to PHA-stimulated PBMCs, MDM, MDDCs and PM-1 T cells overnight. Virus was washed off and cells were cultured for 14 days. Viral replication was assessed by HIV-1 p24 activity and the $TCID_{50}$/ml were calculated. The $TCID_{50}$/ml on TZM-bl cells was determined by adding serial dilutions of the viruses on the cells for 24 hours. Cells were washed and lysed before luciferase expression was assessed using a luciferase reporter assay.

HIV-1 competitions in explant tissue: Cervical or penile tissue explants were exposed to mixtures of acute and chronic viruses for 3 hours at 37° C. After washing, the tissue was incubated in fresh media for another 24 hours. Subsequently migratory cells (MC) were collected and co-cultured with PM-1 T cells. Both, tissue and migratory cells co-cultured with T cells were cultured for 12 days and supernatant was harvested every 3 days. Tissues and cells were lysed for PCR amplification of proviral DNA and 454 pyrosequencing.

454 Sequencing: Fragments flanking the C2V3 env region of HIV-1 were PCR amplified by nested PCR reactions and barcoded with 454 primers. The amplified products were pyrosequenced on a 454 GS Junior instrument. Sequences were binned based on the barcode, aligned and then quantified for each isolate via phylogenetic alignments with the input virus sequence.

References

Development of a yeast-based recombination cloning/system for the analysis of gene products from diverse human immunodeficiency virus type 1 isolates. Marozsan AJ., Arts EJ. J Virol Methods2003 Aug;111(2):111-20.

Mucosal tissue tropism and dissemination of HIV-1 subtype B acute envelope-expressing chimeric virus. King DF., Siddiqui AA., Buffa V., Fischetti L., Gao Y., Stieh D., McKay PF., Rogers P., Ochsenbauer C., Kappes JC., Arts EJ., Shattock RJ. J. Virol 2013 Jan;87(2):890-9.

Results

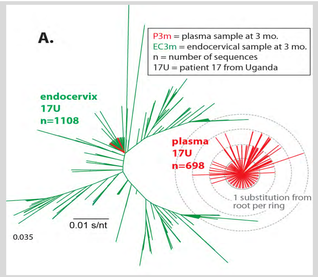


Fig.1: HIV-1 diversity in the female genital tract and blood during acute infection. Plasma and endocervical samples 3 month after infection were analyzed for genetic diversity. Acute viruses from the endocervix showed high genetic diversity while isolates from matched blood were genetically homogenous.

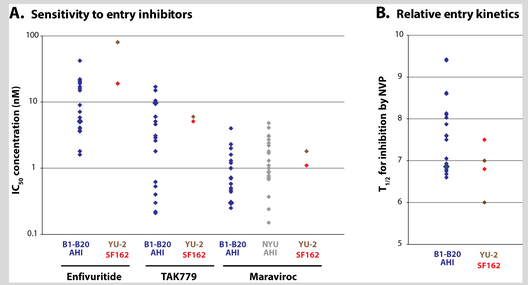


Fig.2: Acute and chronic Env-chimeric viruses display similar A) sensitivity to entry inhibitors and B) viral entry kinetics.

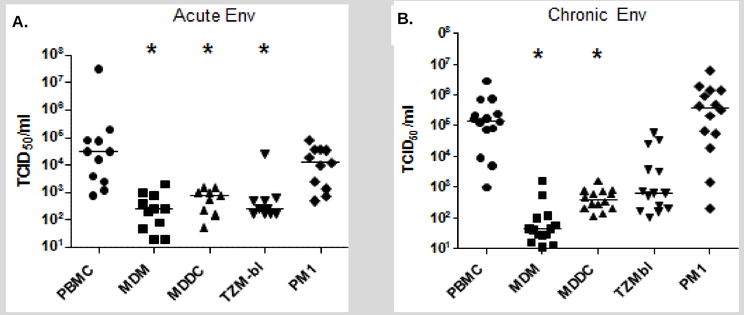


Fig.3: Replication kinetics of Env-chimeric isolates in different cell types. Replication fitness of acute and chronic Env-chimeric viruses was assessed in PBMCs, MDM, MDDCs, TZM-bl and PM-1 cells. Both, A) acute and B) chronic viruses displayed similar replication ability across the tested cell types.

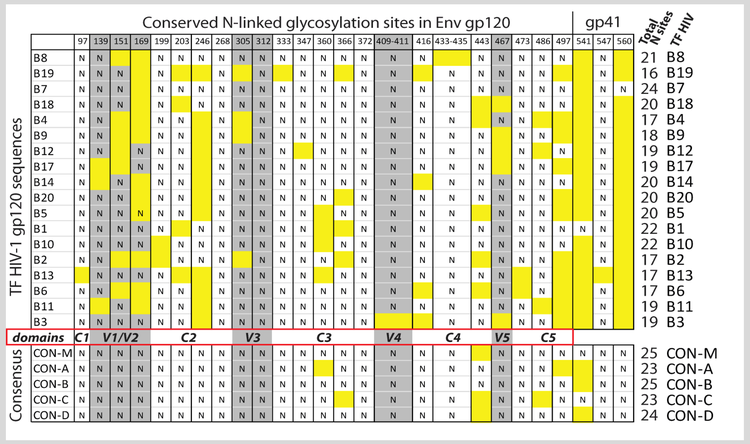


Fig.4: N-linked glycosylation sites on HIV-1 env. Sequence analysis of full length HIV-1 env showed reduced numbers of key conserved N-linked glycosylation sites in env of acute isolates (B1-B20).

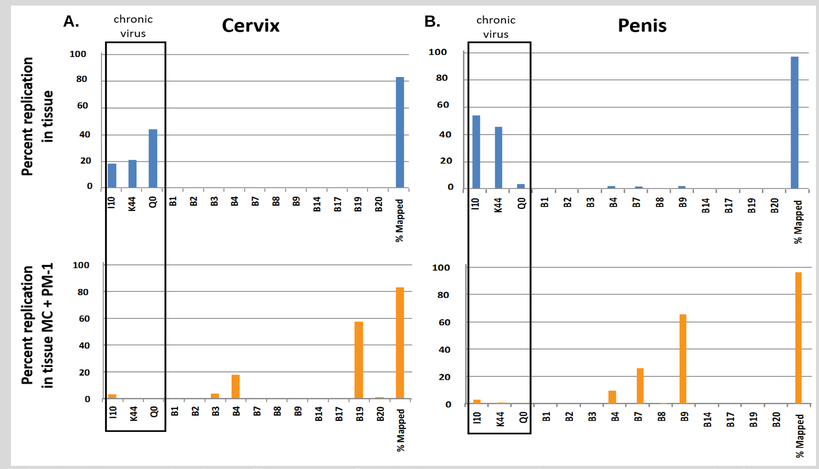


Fig.5: Transmission fitness of HIV-1 across human mucosal tissue. Analysis of mixed competitions of acute and chronic Env-chimeric viruses in A) cervical and B) penile tissue showed that chronic viruses (I10, K44, Q0) primarily replicated in the tissue (top panels), while acute viruses (B1-B20) preferentially penetrated the tissue and are transmitted to T cells (bottom panels).

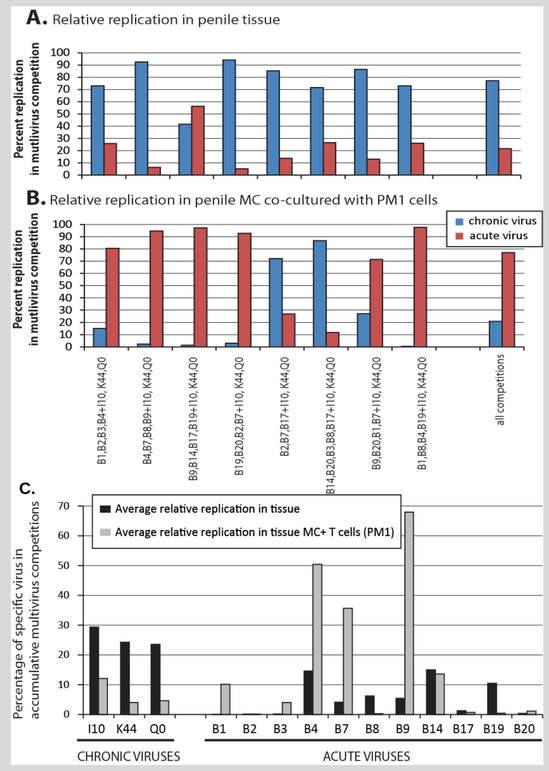


Fig.6: Summary of transmission fitness in mixed virus infections of penile tissue. The percent relative replication of acute (red) and chronic (blue) viruses in multivirus competitions in A) penile tissue and B) in penile tissue MC co-cultured with PM-1 T cells are shown. C) Analysis of the average relative transmission fitness of individual chronic and acute viruses in accumulative multivirus competitions in penile tissue.

Conclusion

Chronic HIV-1 isolates appear to stay and replicate in the mucosal tissue, while acute isolates are preferentially bound by tissue residing DCs/LCs and are subsequently transmitted to T cells. High levels of extracellular soluble lectins in tissue and C-type lectins expressed on epithelial cells are thought to prevent infection of high mannose-containing pathogens. As such, HIV-1 with a reduced glycan shield may be passively selected for transmission by escaping the “lectin-trap” due to reduced binding ability.