HIV Recombination in the In Vitro T_{CM} Latency Model – Reasons and Solutions

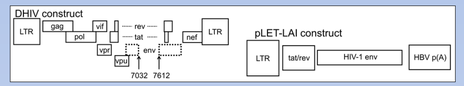
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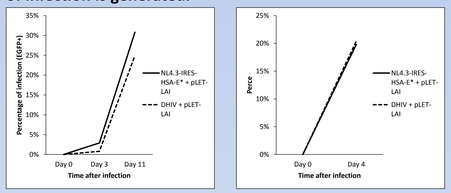
Replication with *env*-deficient constructs

Co-transfection of env-deficient HIV with env leads to replication

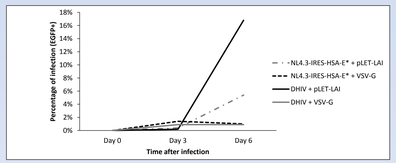
The T_{CM} model described by Bosque and Planelles in 2009 [1] generates large numbers of latently infected cells with memory phenotype. Recent experiments revealed high levels of background infection and massive cell death, reminiscent of a spreading infection, despite the use of a single-round viral vector.



Replication deficient virus is generated by co-transfection of the DHIV constructs with a 600 bp deletion in Env and the pLET-LAI construct, consisting of a wild type env sequence. This way a vector capable of only a single round of infection is generated.



Primary infection (left) of Jurkat and T_{CM} leads to increasing levels of EGFP signal, suggesting spreading infection. Secondary infection (with the supernatant from infected cells; right) of Jurkat indicates that infectious virus is released into the culture medium despite using the *env* deficient DHIV construct. Similar results were observed using an *env* deficient construct with a frameshift mutation in *env* (i.e. NL4.3-IRES-HSA-E*).

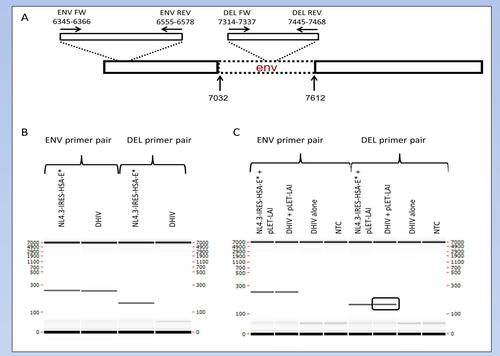


Experiments with VSV-G pseudotyped DHIV lead to stable levels of infection excluding contamination and hinting recombination between pLET-LAI and DHIV as the source of replication competent virus.

Recombination leads to replication

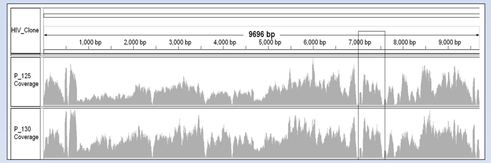
Recombination between overlapping sequences of env leads to HIV replication

PCR and deep sequencing experiments indicate the presence of the deleted env sequence integrated in the genome of cells infected with DHIV.



Using two sets of primer pairs aligning in the env region, we show that the deleted env sequence in DHIV is present in the cells infected by DHIV co-transfected with pLET-LAI.

A. Primer pair ENV aligns to the common sequence of env of DHIV and of the full length env, primer pair DEL aligns to the intact env sequence within the deleted part (dotted line) of DHIV. **B-C**. Electrophoretic separation of PCR products performed on plasmid DNA-NL4.3-IRES-HSA-E* and DHIV **(B)** and on DNA from cells infected with the indicated viruses **(C)**. The positive signal in cells infected with DHIV + pLET-LAI indicates the presence of a full length env sequence in the viral DNA.



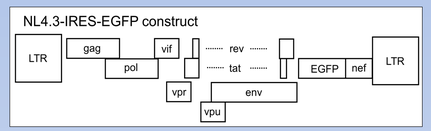
RNA reads aligned in the region of the missing fragment of *env* of the *env*-deficient backbone. A large number of reads aligned in the supposedly deleted fragment of *env* indicates the restoration of the full gene sequence by recombination.

Modified model

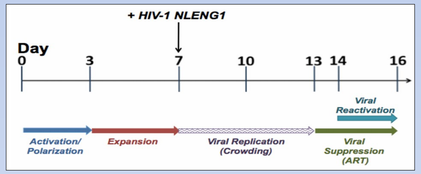
Replication-competent NL4.3 HIV in combination with antiretroviral blockage of HIV replication leads to reproducible levels of latency formation

To avoid stochastic recombination events, a true replication-competent virus was introduced. The implementation of a replication-competent NL4.3 HIV in combination with antiretroviral blockage of HIV replication proved a more reproducible method to generate HIV latency in the T_{CM} model. This new approach more closely mimics the in vivo scenario. Reporter genes enable for sorting out of productively infected cells and facilitate the readout in the model.

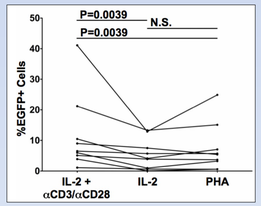
A. HIV reporter approach



Replication-competent construct used to establish HIV latency in the new T_{CM} model. EGFP facilitates the practical work with the model.

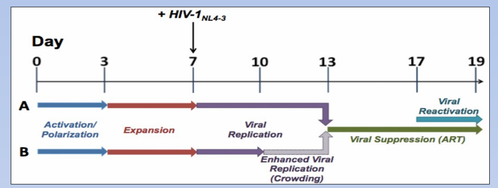


The workflow involves infection of central memory T cells at day 7 after isolation, ARV treatment at day 13 followed by activation to reveal latency and the flow-cytometric readout the following day.

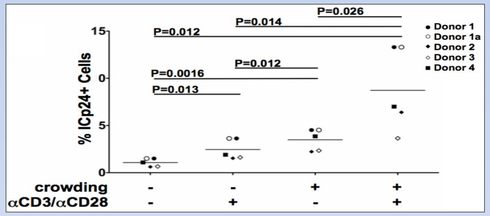


The effect of CD3/CD28 (left) and PHA (right) stimulation used to reveal latency levels in the model compared to the baseline levels of non-stimulated cells (middle). A significant increase indicative of latent infection was observed in cells from all the donors used in the study with CD3/CD28 activation.

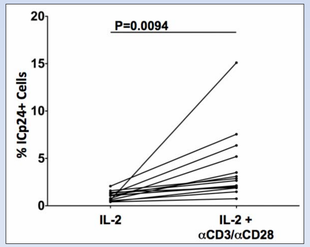
B. Cell crowding approach



The workflow with or without cell crowding (increasing the cell density by culturing them in a round bottom plate). Crowding from day 10 until day 13 influences the levels of latency in the model most.



The influence of crowding on the levels of active and latent infection (as revealed by CD3/CD28 stimulation) in the model.



Activation of central memory T cells leads to increased levels of p24 indicative of reversible latent infection.

Conclusions

- Recombination between overlapping sequences of the *env*-deficient backbone and full-length *env*, supplied in trans, leads to the generation of replication-competent viral particles
- Experiments utilising non-HIV envelope (VSV-G) showed no signs of recombination
- PCR and next generation sequencing confirmed the presence of an intact *env* gene in infected cells
- A replication-competent HIV-1 strain was implemented into the model in combination with antiretroviral blockage of viral replication to generate latently infected cells
- EGFP/HSA reporter genes facilitate the tracking of infection levels and the readout, they also enable for the sorting of productively infected cells
- The new approach utilising replication-competent viruses leads to significant level of latency in central memory T cells

References and acknowledgements

1. Bosque 2009, Blood; 113(1): 58–65.
2. Bonczkowski 2014, Retrovirology; 11:70
3. Martins 2015, manuscript submitted.

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