Construction and Characterization of two Chimeric HTLV-1$_{AC}$ infectious Molecular Clones

Sarkis S[1], Moles R[1], Gutowska A[1], Galli V[1], Omsland M[1], Washington-Parks R[1], Purcell DFJ[2], Pise-Masison C[1], Franchini G[1]

[1]*Animal Models and Retroviral Vaccines Section, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
[2]*Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, VIC, Australia.*

Background:
HTLV-1C, the most divergent virus variant, has recently is endemic in indigenous populations in Central Australia. HTLV-1 A and C apparently differ in their clinical manifestation as well as in the *orf-I* sequence. Given the importance of *orf-I* expression for HTLV-1A fitness, we investigated whether and how *orf-I* is expressed in HTLV-1C infection and investigate whether HTLV-1A and HTLV-1C infection causes different inflammatory profiles *in vitro* and in animals *in vivo*.

Methods:
We engineered two chimeric HTLV-1$_{A/C}$ molecular clones by inserting into the pAB HTLV-1A backbone either the HTLV-1C *orf-I, II* (HTLV-1$_{ACO-I/II}$) or *orf-I, II, III*, *IV* genes and the *3'LTR* (HTLV-1$_{ACO-L}$).

Results:
We found that that un-spliced, singly and doubly spliced mRNAs identified in HTLV-1A are present in cells transfected with both chimeric molecular clones and demonstrated that subtype C *orf-I* is expressed via a doubly spliced mRNA that juxtaposes the first exon of *rex,* and its ATG in frame to *orf-I.* This *mRNA* encodes a 16KDA protein (p16). Western blotting further demonstrated the presence of HTLV-1 p24Gag, gp46Env and Tax protein in both transfected cells as well as in stably infected 729.6 B cells producing the chimeric viruses. Similar, to HTLV-1A, the Tax protein encoded by HTLV-1$_{ACO-L}$ is a potent activator of CREB/ATF and of NF-kB. Moreover, following the co-cultivation of HTLV-1$_{ACO-I/II}$ and HTLV-1$_{ACO-L}$ infected 729.6 B cells with the SupT-1-LTR-GFP reporter cells we demonstrated that both chimeric viruses can be transmitted to human CD4+T-cells.

Conclusion:
Our data demonstrate that two HTLV-1$_{AC}$ chimeric molecular clones, whereby either the type C *orf-I/II*, or all 3'*orfs* and LTR, were swapped into HTLV-1A, are biologically active and infectious. The availability of these molecular clones, hopefully, will provide the opportunity to study HTLV-1C pathogenicity and inflammatory profile in macaques, a relevant animal model for testing approaches to prevent infection and treat diseases associated with HTLV-1C infection