Construction and Characterization of two Chimeric HTLV-1\textsubscript{AC} Infectious Molecular Clones

Sarkis S\textsuperscript{1}, Moles R\textsuperscript{1}, Gutowska A\textsuperscript{1}, Galli V\textsuperscript{1}, Omsland M\textsuperscript{1}, Washington-Parks R\textsuperscript{1}, Purcell DFJ\textsuperscript{2}, Pise-Masison C\textsuperscript{1}, Franchini G\textsuperscript{1}

\textsuperscript{1}Animal Models and Retroviral Vaccines Section, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
\textsuperscript{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 \textit{in vitro} and in animals \textit{in vivo}.

Methods:
We engineered two chimeric HTLV-1\textsubscript{AC} molecular clones by inserting into the pAB HTLV-1A backbone either the HTLV-1C orf-I, II (HTLV-1\textsubscript{ACO-I/II}) or orf-I, II, III, IV genes and the 3’LTR (HTLV-1\textsubscript{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\textsubscript{ACO-L} is a potent activator of CREB/ATF and of NF-kB. Moreover, following the co-cultivation of HTLV-1\textsubscript{ACO-I/III} and HTLV-1\textsubscript{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\textsubscript{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.