**HTLV-1C lung disease linked to p16 protein inhibition of efferocytosis in macaques**

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**Abstract:**

Background: HTLV-1A is the most common type worldwide, and HTLV-1C, the most divergent variant, is endemic among Aboriginal populations in the Northern Territory, Australia, with a seroprevalence above 30%1. Clinical data from this population suggest a higher association of HTLV-1C infection with respiratory failure and premature death; contrary to type A rarely associated with lung disease, possibly stemming from a genetic difference in the HTLV-1 subtypes2,3. The highest nucleotide divergence in the genomes of HTLV-1 A and C occurs at the 3’end of the virus specifically in *orf-I*, encoded by a singly spliced *mRNA* in HTLV-1A. While *orf-I* expression is central to HTLV-1A infectivity, early findings that HTLV-1C lacks the AUG initiation codon for *orf-I* translation have recently been corroborated, leading us to hypothesize that HTLV-1C expresses *orf-I* by an alternative mechanism4.

Materials and Methods: To test our hypothesis, we constructed a chimeric HTLV-1A/C molecular clone *in silico* by cloning the entire *orf-I*, the overlapping *orfs II*, *III, IV,* and the 3’LTR of HTLV-1C derived from an infected human patient into the HTLV-1A molecular clone backbone and tested its infectivity in human and macaque CD4+ T-cells *in vitro* and in macaques *in vivo.*

Results: We found that subtype C *orf-I* (*orf-IC*) can be expressed *in vitro* and *in vivo* in the lungs of infected macaques by a doubly spliced mRNA juxtaposed with the first exon of *rex,* which provides its ATGin frame with *orf-I* and encodes the p16C protein (*rex-orf-IC*). Although the chimeric HTLV-1A/C virus exhibited similar infectivity to HTLV-1A in macaques, the host inflammatory response in blood and bronchoalveolar lavage (BAL) differed significantly. Interestingly, HTLV-1A/Cinfection of lung tissue was associated with infiltrates of T-cells and B-cells as well as neutrophils and monocytes/macrophages producing IL-8 and TNF-α with interstitial pneumonia, alveolitis, and bronchiectasis consistent with the development of lung fibrotic disease. In contrast, cell infiltrates in HTLV-1A predominantly expressed IL-10, a pro-resolution cytokine that mitigates inflammation. Moreover, *In vitro,* T-cells expressing p16C become resistant to engulfment by efferocytosis, a monocyte function that clears apoptotic cells and maintains tissue homeostasis.

Conclusion: Our data suggest that p16C expression in lung, mediates the pathogenetic mechanism underlying the increased inflammation and lung disease observed in HTLV-1C infection and pointing to p16 as a possible druggable target to prevent HTLV-1C lung morbidity.

References

1. Einsiedel, L.J., Pham, H., Woodman, R.J., Pepperill, C. & Taylor, K.A. The prevalence and clinical associations of HTLV-1 infection in a remote Indigenous community. *Med J Aust* **205**, 305-309 (2016).

2. Einsiedel, L.*, et al.* Human T-Lymphotropic Virus type 1c subtype proviral loads, chronic lung disease and survival in a prospective cohort of Indigenous Australians. *PLoS Negl Trop Dis* **12**, e0006281 (2018).

3. Einsiedel, L., et al. Predictors of non-cystic fibrosis bronchiectasis in Indigenous adult residents of central Australia: results of a case-control study. *ERJ Open Res* **5**(2019).

4. Gessain, A., Boeri, E., Yanagihara, R., Gallo, R.C. & Franchini, G. Complete nucleotide sequence of a highly divergent human T-cell leukemia (lymphotropic) virus type I (HTLV-I) variant from melanesia: genetic and phylogenetic relationship to HTLV-I strains from other geographical regions. *J Virol* **67**, 1015-1023 (1993).

5. Cassar, O.*, et al.* A Novel Human T-lymphotropic Virus Type 1c Molecular Variant in an Indigenous Individual from New Caledonia, Melanesia. *PLoS Negl Trop Dis* **11**, e0005278 (2017)