The dynamics and consequences of HTLV-1 Tax expression in naturally infected T-cell clones

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Background:
The regulation of proviral expression is essential for HTLV-1 persistence *in vivo*. Plus-strand proviral expression is minimal in freshly isolated HTLV-1-infected T lymphocytes, but short-term *in vitro* culture leads to intense plus-strand transcriptional bursts. Previous single-cell studies on HTLV-1 expression have used either single-molecule RNA-FISH, which requires cell fixation and therefore loses temporal information or transformed cell lines containing multiple proviral copies, unlike naturally infected cells. Here we report the dynamics of HTLV-1 Tax expression in naturally infected, non-transformed T-cell clones at single-cell resolution and the consequences of Tax expression in these cells.

Methods:
We used two non-transformed T-cell clones, each carrying a single copy of the HTLV-1 provirus at a mapped integration site. To visualise the dynamics of Tax expression in viable cells, we stably transduced the clones with a Tax reporter cassette containing a short half-life enhanced GFP (d2EGFP) gene downstream of 18 tandem Tax responsive elements.

Results:
Live-cell imaging followed by semi-automated single-cell tracking revealed several distinct patterns of Tax expression within each clonal population. Most (50% to 66%) Tax-expressing cells continued to express for at least 30 hours. Tax expression was associated with decreased proliferation (Ki-67), slower cell-cycle progression, increased DNA damage (γH2AX) and increased risk of apoptosis (Annexin-V). Flow-sorting and subsequent serial flow cytometry analysis showed enhanced proliferation in flow-sorted Tax^+ cells after they had terminated Tax expression, resulting in a greater increase in cell count in the flow-sorted Tax^+ population after 14 days' culture.

Conclusion:
These results quantify the dynamics and clonal heterogeneity of HTLV-1 proviral expression in naturally-infected cells. The data suggest that a post-Tax expression proliferative burst occurs to compensate for the detrimental effects of long Tax bursts in naturally-infected T-cell clones.

Disclosure of Interest Statement:
None