Can thymic epithelial cells be infected by human T-lymphotropic virus type 1?

Klaysia Moreira-Ramos1,2,3, Flávia Madeira Monteiro de Castro1, Leandra Linhares-Lacerda1, Wilson Savino1/+  

1Laboratório de Pesquisas sobre o Timo, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21040-360 Rio de Janeiro, RJ, Brasil  
2Programa de Pós-Graduação em Oncologia, Instituto Nacional do Câncer, Rio de Janeiro, RJ, Brasil  
3Universidade Estadual de Ciências da Saúde de Alagoas, Maceió, AL, Brasil

The human T-lymphotropic virus type-1 (HTLV-1) is the cause of adult T cell leukaemias/lymphomas. Because thymic epithelial cells (TEC) express recently defined receptors for the virus, it seemed conceivable that these cells might be a target for HTLV-1 infection. We developed an in vitro co-culture system comprising HTLV-1+-infected T cells and human TECs. Infected T cells did adhere to TECs and, after 24 h, the viral proteins gp46 and p19 were observed in TECs. After incubating TECs with culture supernatants from HTLV-1+-infected T cells, we detected gp46 on TEC membranes and the HTLV-1 tax gene integrated in the TEC genome. In conclusion, the human thymic epithelium can be infected in vitro by HTLV-1, not only via cell-cell contact, but also via exposure to virus-containing medium.

Key words: thymic epithelial cells - HTLV-1 infection - adult T cell leukaemia/lymphoma - cell adhesion
virus particles. We then plated 5 x 10^5 TECs in culture membranes, which retain cells but allow the passage of supernatants were filtered through 0.22 µM pore size and fixed in 1% formaldehyde (Ribeiro-Carvalho et al. 2002). Image acquisition and analysis were performed using a FACSCalibur® device (Becton Dickinson) equipped with CellQuest software.

To determine whether viral proteins could be detected in TECs, in some experiments, TECs and C91PL cells were co-cultured for 24 h and then submitted to double-label immunohistochemistry, as described previously (Lepelletier et al. 2007). For this experiment, we used polyclonal pan-cytokeratin rabbit serum (Dako Co, Carpinteria, USA), anti-HTLV-1 gp46 and anti-HTLV-1 p19 (Abcam) monoclonal antibodies, as well as the secondary antibodies Alexa-488-labelled goat anti-mouse and Alexa-546-coupled goat anti-rabbit Ig (Amersham Biosciences, Buckinghamshire, UK). TECs were cultured for 24 h in Lab-Tek chambers and were sequentially subjected to a given primary monoclonal antibody and the corresponding fluorochrome-labelled antibodies for 20 min, washed in PBS and fixed in 1% formaldehyde. TECs were co-cultured for 24 h and then the TECs were subjected to a given primary monoclonal antibody and the corresponding fluorochrome-labelled antibodies for 20 min, washed in PBS and fixed in 1% formaldehyde (Ribeiro-Carvalho et al. 2002). Image acquisition and analysis were performed using a FACSCalibur® device (Becton Dickinson) equipped with CellQuest software.

Other experiments were designed to determine whether culture supernatants derived from HTLV-1 virus particles can convey viral elements to cultured TECs. For these experiments, lymphocytes (2 x 10^6/mL) were cultured for 72 h in Falcon tubes and centrifuged, and the supernatants were filtered through 0.22 µM pore size membranes, which retain cells but allow the passage of virus particles. We then plated 5 x 10^5 TECs in culture flasks. Twenty-four hours later, these cells were incubated with supernatant harvested from infected T cells cultured for 72 h. Cells were then washed, immunostained with the anti-gp46 antibody to detect this viral protein on their surface and analysed by cytofluorometry. As shown in Fig. 2C, only TECs incubated with supernatants from HTLV-1-infected lymphocytes were labelled to visualise viral gp46.

Finally, we used reverse transcription-PCR to screen for the presence of the Tax gene in the genome of TECs treated with HTLV-1 infected cells. These experiments revealed that HTLV-1 was able to penetrate into TECs and integrate its proviral DNA (Fig. 2D).

For the various experiments summarised above, quantitative data were expressed as the mean ± standard error and the results were statistically analysed using Student t test. Differences were considered statistically significant when the p values were < 0.05.

In the first set of experiments, we showed that after 2 h co-cultures, infected T cells were able to adhere to TECs, exhibiting an AI that was significantly higher than that obtained with the uninfected T cells (Fig. 1). We thus tested whether, in heterocellular cell-cell adhesion conditions, we could detect viral proteins in TECs. We co-cultured the cells for 24 h and then the TECs were stained with anti-cytokeratin and anti-gp46 antibodies. As seen in Fig. 2A, viral gp46 and cytokeratin were co-localised in TEC cultures, indicating that these cells might have HTLV-1 on their surface or in the cytoplasm. This result was confirmed using double-label immunofluorescence for the detection of cytokeratins and the viral matrix protein p19 (Fig. 2B).

We then tested the hypothesis that TECs can be infected by free HTLV-1 virions using the supernatant of infected T cells. Epithelial cells were cultured for 90 min with supernatant harvested from infected T cells cultured for 72 h. Cells were then washed, immunostained with the anti-gp46 antibody to detect this viral protein on their surface and analysed by cytofluorometry. As shown in Fig. 2C, only TECs incubated with supernatants from HTLV-1-infected lymphocytes were labelled to visualise viral gp46.
In conclusion, we showed herein that the human thymic epithelium can be infected in vitro by HTLV-1, not only via cell-cell contact, but also via exposure to virus-containing medium.

Given that activated T lymphocytes and dendritic cells recirculate from the periphery to the thymus (Tian et al. 2007, Li et al. 2009) and that HTLV-1 can be transmitted in a cell-free manner, as has been reported for dendritic cells (Jones et al. 2008), it is conceivable that T lymphocytes and dendritic cells, once infected by HTLV-1, carry the virus into the thymus and infect microenvironmental cells, such as TECs. Thus, we hypothesize that the human thymic epithelium is a putative reservoir for HTLV-1, opening up a research field related to the role of the thymus in the HTLV-1 infection cycle.

REFERENCES


