

Study of the mechanism of mother-to-child transmission of simian T-cell leukemia virus type 1 in Japanese macaques

Poonam

Background: Simian T-cell leukemia virus type 1 (STLV-1) is a simian orthologue of human T-cell leukemia virus type 1 (HTLV-1) that may cause neuromyelopathy and adult T-cell leukemia/lymphoma (ATLL) among some of the infected individuals. Both viruses can be transmitted between individuals through exposure to bodily fluids containing infected cells. The primary transmission routes include mother-to-child transmission (MTCT) through prolonged breastfeeding, sexual transmission through semen, and exposure to infected blood. In humans, MTCT frequency is estimated to be around 20% and almost all ATLL cases originate from MTCT. Therefore, prevention of MTCT is essential to inhibit the disease onset. Current strategies to prevent MTCT include stopping breastfeeding entirely or limiting it to less than three months. However, these strategies cannot be implemented globally, and there is a need for novel strategies to prevent HTLV-1 MTCT. This requires an in-depth understanding of the mechanism of HTLV-1 MTCT, which remains largely unknown to date. In the present study, I aimed to understand the mechanism of STLV-1 MTCT in Japanese macaques (JMs), which may provide valuable insights into HTLV-1 MTCT.

Methods: Several lactating JM mothers and their infants were employed in the study. For analysis of milk-infected cells, paired blood and breast milk samples were obtained for JMs. Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood through density gradient centrifugation. The proportions of lymphocytes in PBMCs and breast milk cells (BMCs) were confirmed by flow cytometry. STLV-1 proviral loads (PVLs) and the mRNA expression of STLV-1 genes were quantified using quantitative and real-time PCR, respectively.

For the longitudinal analysis of STLTV-1 infection status in infants, stored PBMC samples obtained from nine infants at every three months after birth until three years of age were analyzed for measurement of STLTV-1 PVLs using quantitative PCR. The STLTV-1 *env* gene sequence of mother-infant pairs was analyzed via Sanger sequencing. STLTV-1 clones in the PBMCs of mother-child pairs were identified and analyzed using next-generation sequencing.

Results: In the present study, I attempted to understand the mechanism of MTCT of STLTV-1 using STLTV-1-infected JMs from the mothers' side, specifically focusing on how milk-infected cells may transmit the virus to infants (chapter 2) as well as from the infants' side (chapter 3), by sequential analysis of the infection status of STLTV-1 in the infants. Since there was no information available regarding the virus in the breast milk of STLTV-1-infected NHPs, I first optimized the process of milk collection from lactating JMs (Chapter 2). Next, I investigated whether STLTV-1-infected cells could be detected in the breast milk of JMs, and if so, is there a link between infected cells in milk and those in peripheral blood? I found that almost all the infected JM mothers contained STLTV-1-infected cells in their breast milk, and the frequency of infected cells was comparable between milk and blood. Next, I investigated what features of milk-infected cells could potentially lead to infection in infants if ingested through breastfeeding. To test this, I analyzed whether milk-infected cells can be induced to express *tax* mRNA via short-term culture stimulation. This is important because *tax* gene expression, leading to expression of Tax protein, is essential for the infected cells to transmit the virus to nearby uninfected cells. I found that breast milk cells (BMCs) of all the STLTV-1-infected JM mothers showed *tax* mRNA expression after 1-day culture, suggesting that all the JM mothers may have the potential to infect their children via breastfeeding. Interestingly, I also found that around 20% of the carrier JM mothers exhibit viral *tax* mRNA expression (*in vivo*) in their BMCs, but rarely in PBMCs.

Moreover, I found that mothers with *in vivo* expression of *tax* mRNA in their BMCs had significantly higher PVLs compared to the JM mothers showing no such expression, suggesting the relationship between the two.

In chapter 2, I found that all the JM mothers may have the potential to infect their children via breastfeeding based on the *tax* mRNA expression via culture stimulation. Considering this, it is reasonable to assume that all the infants born to carrier mothers could be at risk of developing infection if breastfed continuously. However, it is well known that only a fraction (~20%) of infants become infected, based on the prevalence of PTLV-1s among young infants. Alternatively, is it safe to assume that the remaining 80% of infants never became infected despite continuous milk feeding? To address these questions, I sought to sequentially examine infants born to STL-1-infected JM mothers and thoroughly understand their infection status (chapter 3). Fortunately, a longitudinal study of 9 mother-child pairs had been ongoing for the purpose of assessing MTCT frequency among JM infants in my lab. This study involved measurement of PVLs in PBMCs of infants at every three months starting immediately after birth up to three years of age. Continuing with the study, I found that around 5 out of 9 (55%) infants were STL-1-positive during the milk feeding duration. Surprisingly, during subsequent analysis, only 2 of these 5 infants maintained persistent STL-1 infection at least until 3 years of age. While the remaining three infants who were positive for provirus at 5 and 8 months of age were found to be consistently negative for STL-1 thereafter, at least until three years of age. These three infants seemed to be transiently infected with STL-1, which was very surprising considering that retroviruses are known to infect lifelong. Through various experiments, I confirmed that these three infants were indeed infected with STL-1 during early infancy; however, they managed to control viral growth

to undetectable levels later on. These infants represent a very unique phenomenon of natural remission of retroviral MTCT, observed for the first time among entire retroviruses.

Discussion and conclusion: This study aimed to understand how STLV-1 spreads from mothers to their children in JMs. A key finding emerged from analyzing breast milk samples. We observed that mothers exhibiting high PVLs in their milk frequently displayed active *tax* gene expression within their BMCs. This *in vivo* activity strongly suggests a direct capacity for viral transmission during breastfeeding. These findings suggested a clear link between high PVL and *tax* gene expression in the milk. Furthermore, a more detailed analysis revealed a clear correlation between maternal viral load and the outcome of infant infection. Specifically, mothers whose infants developed persistent STLV-1 infections showed significantly higher PVLs in their PBMCs (which is highly correlated with PVL in the breastmilk) compared to mothers of infants who experienced only transient infections or remained uninfected. Taken together, these findings suggest that the expression of the *tax* gene in the breast milk of STLV-1 carrier mothers may be a potential indicator for the establishment of MTCT. These results may be extrapolated to HTLV-1 MTCT and could assist in developing future prevention strategies.