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－Review Article－

Milk-Borne Transmission of Human T-Cell Lymphotropic Virus Type I (HTLV-I) and Its Intervention in Nagasaki

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Summary

Human T-cell lymphotropic virus type I (HTLV-I), a causative virus of adult T-cell leukemia (ATL), transmits through both horizontal and vertical pathways. Since ATL develops in only carriers infected early in life, vertical transmission cycle is the most important target of intervention for the purpose of the prevention of ATL. Epidemiological studies in an endemic area, Nagasaki, Japan, and animal experiments identified milk-borne maternal transmission as the major vertical pathway. The prefecture-wide intervention in Nagasaki since 1987 has revealed that the refraining from breast-feeding by carrier mothers can prevent about 85% of maternal HTLV-I transmission. Pathways for the remaining 15% await for elucidation. However, our studies argued against the possibilities of intrauterine transmission and infection via saliva. Perinatal transmission remains to be evaluated as the alternative pathway. At present, refraining from breast-feeding is the most effective measure to break the cycle of maternal HTLV-I transmission in endemic areas. It is estimated that the intervention in Nagasaki for the past 10 years has prevented 1,000 maternal transmission and 50 future ATL cases. I am reasonably confident that incidence of ATL in Nagasaki will decline to the national average level over the next few generation if the intervention program stays alive.

Key words: HTLV-I, maternal transmission, intervention

Introduction

Human T-cell lymphotropic virus type I (HTLV-I), a human retrovirus, is a causative agent of adult T-cell leukemia (ATL)[1,2] as well as HTLV-I-associated myelopathy／tropical spastic paraparesis (HAM／TSP)[3,4] and some other inflammatory disorders of various organs[5-8]. HTLV-I is endemic in the specific geographic areas in the world, such as Japan, Caribbean basin, South America, and Africa[9-12]. In Japan, the virus is distributed in southwestern coast[9], including Nagasaki Prefecture located in the westernmost part of mainland Japan. Carriers of HTLV-I in Nagasaki have been estimated to be about 60,000 (4%) in a population of 1.5 million[13]. The prevalence of HTLV-I carriers increases with age: the average prevalence among those older than 40 years is about 10%[13]. Approximately 4-5% of carriers are presumed to develop ATL after a long latency. The annual incidence of ATL in Nagasaki is about 100 cases, which corresponds to 0.5% of total death in the prefecture[14,15]. ATL is a fatal disease due to its resistance against a conventional chemotherapy, high incidences of hypercalcemia, and leukemic cell infiltration in various organs. Unfortunately, there is no measure to prevent the development of ATL among carriers at present. Breaking the cycle of HTLV-I transmission has thus been urgently needed in the endemic areas.

HTLV-I infects mainly CD4+ T cells in vivo and the proviral genome is integrated in chromosomal DNA of the infected cells. The virus transmits through both horizontal and vertical pathways. Since HTLV-I infects in only cell-to-cell manner[16], the sources of infection are required to contain a significant number of infected T cells. The major routes of horizontal transmission are through blood transfusion and sexual contact. About a half of recipients transfused with carriers' whole blood were shown to be infected[16]. In Japan including Nagasaki, however, the screening of donated blood for antibodies to HTLV-I since 1985 has reduced the blood-borne infection to the negligible level[17]. Sexual transmission via seminal fluids was proved by the demonstration of HTLV-I-infected cells in carriers' seminal fluids[18] and by the epidemiological data

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showing that most of the wives of carrier husbands were infected in a Japanese cohort[19]. However, one-way male-to-female transmission is not always the case in other population. Female-to-male transmission is also frequent in countries where other sexually transmitted diseases (STD), including human immunodeficiency virus (HIV) infection, are common[20]. These diseases themselves, resulting immunosuppression, and/or promiscuous sexual behaviour of STD patients may increase vulnerability to sexual HTLV-I infection. Sexual transmission, together with maternal transmission detailed in the following sections, are likely to have resulted in the familial clustering of carriers, a characteristic feature of HTLV-I endemicity.

HTLV-I exerts its pathogenicity in only a minor population of carriers. Although pathogenic mechanisms are not well understood, epidemiological studies have identified some risk factors for some HTLV-I-associated diseases. For instance, HTLV-I infection through blood transfusion was shown to be a risk factor for HAM/TSP[21]. In contrast, ATL seems to develop in only carriers infected early in life[22,23], since there is no confirmed ATL cases among carriers infected through blood-borne or sexual transmission to date. This may be due to the multi-step leukemogenesis of ATL which requires a long latency, usually 40-50 years after infection[24]. Maternal infection cycle is thus the most important target of intervention for the purpose of the prevention of ATL. In this review article, I introduce the studies conducted at Nagasaki University School of Medicine for last 15 years regarding the identification of breast milk as the major source of maternal HTLV-I transmission, the intervention of the milk-borne transmission in Nagasaki, and the attempts to identify alternative maternal pathways.

Maternal transmission of HTLV-I via breast milk

The presence of mother-to-child pathways of HTLV-I transmission was first indicated by the epidemiological studies in an endemic area, Nagasaki, in 1985. Hino et al.[13] demonstrated that HTLV-I seroprevalence among children born to carrier mothers (22%) was significantly higher than that of age-matched general population (about 1%). Moreover, more than 90% of carrier children's mothers were shown to be infected[13]. This close association between the infected mother and child strongly suggested the presence of maternal transmission pathways. Maternal transmission may occur through intrauterine, perinatal, and postnatal routes. HTLV-I infection through blood transfusion usually results in seroconversion within a few months after the transfusion[16]. In contrast, most of carrier children seroconvert betweeen 6 and 12 months of age[13]. This delayed seroconversion in carrier children raised the idea that the postnatal pathway is the most probable candidate for maternal HTLV-I transmission.

Since HTLV-I transmission requires infected T cells, we considered the breast milk, containing 10^6 cells/ml in average, as the most likely source of postnatal transmission. By the short-term culture and subsequent indirect immunofluorescence assay, about 0.1% of cells in the breast milk of carrier mothers were found to be infected[25,26]. If a carrier mother gives her baby 500 ml of breast milk every day for 200 days, 10^8 infected cells are presumed to be transferred to the baby before weaning.

Hino et al.[27] then retrospectively asked 83 carrier mothers, including 17 with infected children, for history of breast-feeding. All of the infected children appeared to be breast-fed, and the carrier rate among breast-fed children was 17/73 (23%). In contrast, none of 10 never-breast-fed children was infected.

In order to examine whether oral inoculation of infected cells has a potential to transmit HTLV-I, animal experiments were conducted using the common marmoset (Callithrix jacchus) which lacked simian T-cell lymphotropic virus (STLV) crossreacting with HTLV-I. First, Yamanouchi et al.[26] inoculated cultured peripheral blood mononuclear cells (PBMC) of ATL patients (7.8×10^7 cells in total by 6 inoculations) into the oral cavity of marmosets using a syringe without a needle to avoid the mucosal injury. In one of the two inoculated marmosets, seroconversion occurred 2.3 months after the first inoculation. HTLV-I infection in the seroconverted marmoset was confirmed by the presence of infected T cells in the PBMC after the short-term cell culture[26]. Next, fresh breast milk samples obtained from carrier mothers were orally inoculated to a marmoset, 200ml in total by 23 inoculations. The marmoset again revealed seroconversion 2.5 months after the first inoculation and was confirmed to be infected by the detection of infected T cells[28].

These findings strongly suggested that breast milk is the major source of maternal HTLV-I transmission. Presumably, infected T cells in the milk migrate to the lymphatic tissues in the oral cavity or the intestine, and transmit the virus to children's T cells in cell-to-cell manner.

Intervention of milk-borne HTLV-I transmission in Nagasaki

In an attempt to break the cycle of mother-to-child

transmission, the intervention program, namely ATL Prevention Program (APP), in Nagasaki Prefecture started in 1987 in collaboration with the prefectural government, Nagasaki Medical Association, Nagasaki University School of Medicine, and Nagasaki Central Hospital[27,29]. The APP has involved the screening out of HTLV-1 carriers from among pregnant women, the refraining from breast-feeding by carrier mothers, and the following up of their children. After obtaining the informed consent, pregnant females have been screened for serum anti-HTLV-I antibodies at the end of the second trimester. Carriers were notified the positive results and advised to refrain from breast-feeding by obstetricians. The children born to the carrier mothers have been followed up serologically at 0.5, 1.0, 1.5, 2.0, and 3.0 years of age by pediatricians in the selected hospitals. All the carrier mothers and their children were coded, and they were interviewed for their feeding methods (bootle feeding or breast feeding and its period) prospectively and/or retrospectively.

So far, 124,387 pregnant women in the prefecture have attended the program for 10 years since its start (1988-1997). Based on the annual number of total birhs in the prefecture, the attending rate is estimated to be more than 70%. Among them 5,699 women (4.3%) were found to be infected. The carrier rate has recently been decreasing from 4.4% in average during 1988-1991 to 3.1% in 1996 and 2.5% in 1997. This decline is presumably due to the cohort effect, however, demographic, social or behavioural factors involved in this remains to be elucidated.

More than 90% of the carrier mothers agreed to refrain from breastfeeding. According to a report by the study group of APP in 1998, their prospective studies indicated that the incidence of transmission among bottle-fed children, 2.7%, was about 1/6 of that among breast-fed children, 16.9%. The results finally confirmed breast milk as the major source of maternal HTLV-I transmission, and indicated that refraining from breastfeeding by carrier mothers is the most effective measure to prevent the transmission. It is estimated that the intervention for the past 10 years has prevented 1,000 maternal transmission and 50 future ATL cases.

Intrauterine transmission of HTLV-I is unlikely

Although the intervention has dramatically reduced the frequency of maternal transmission, about 3% of carrier mothers' children were infected despite bottle-feeding, suggesting the presence of alternative maternal pathways other than via breast milk.

Intrauterine transmission was thought to be the most possible candidate for the alternative pathway. Using a highly sensitive nested PCR capable of detecting a single molecule of the HTLV-I provirus, Kawase et al. screened 717 umbilical cord blood samples from babies born to carrier mothers. Eighteen samples (2.5%) gave positive results[30]. This raised the possibility that these children were infected in utero. We then followed up 7 children whose cord blood was PCR-positive, up to between 24 and 48 months after birth. Surprisingly, none showed serological evidence of HTLV-1 infection (Table 1)[31]. Antibodies to HTLV-1 detected in the sera of three 6-month-old babies were considered to be maternal. Maternal antibodies disappeared thereafter and the children older than 12 months were exclusively seronegative. The HTLV-1 proviral DNA in PBMC was also examined at least once for all the children but one by the nested PCR for both gag and pX regions of the HTLV-1 genome. All the results of PCR were negative. Therefore, the presence of HTLV-I in the cord blood is not a hallmark of intrauterine infection. HTLV-I detected in the cord blood was presumably derived from migrated maternal cells and excluded from the circulation after birth. Infected cells in the fetal and baby's circulation seemed to be no longer infectious. Maternal antibodies to HTLV-I in the circulation during pregnancy and for several months postpartum might prevent the HTLV-I transmission.

Next, to determine whether the approximately 3% of bottle-fed children of carrier mothers who were infected with HTLV-1 were infected in utero, we retrospectively examined the presence of HTLV-1 proviral DNA in frozen stocks of their cord blood (Table 2).

Table 1. Follow-up of children whose umbilical cord blood samples were positive for HTLV-I proviral DNA.

Cases	Cord Blood PCR gag	pX	Nutrition	Follow-Up (m., months) Serum Anti-HTLV-1 Antibody 6m.	12m.	18m.	24m.	36m.	48m.
1	+	+	Formula		−		−	−	− (PCR−)
2	+	+	Formula	+	−	− (PCR−)	−		− (PCR−)
3	+	+	Formula	−	−		− (PCR−)	−	
4	+	+	Formula	−	−		−	−	
5	+	+	Formula	+ (PCR−)	−	(PCR−)	−	−	
6	+	+	Formula	+		− (PCR−)	−	−	
7	+	+	Formula				− (PCR−)		
8	+	+	Breast	+		−			

Table 2. Absence of HTLV-I proviral DNA in umbilical cord blood samples of newborns who were formula (bottle)-fed and later confirmed to be infected with HTLV-I.

Cases	Cord Blood PCR gag	pX	Nutrition	Follow-Up (m., months) Serum Anti-HTLV-1 Antibody 6m.	12m.	18m.	24m.	36m.
1	−	−	Formula		+			+
2	−	−	Formula	−			+	+
3	−	−	Formula	+	+	+	+	+
4	−	−	Formula	+	+		+	
5	−	−	Formula	+	+ (PCR−)	+	+	
6	−	−	Formula			+	+ (PCR−)	
7	−	−	Formula	−	+	+	+	
8	−	−	Formula	−	+			
9	−	−	Formula	−	+			

All of 9 tested children were bottle-fed and seropositive at the age of 12 months. Three and 4 of them continued to be seropositive up to 36 and 24 months, respectively. Although data beyond 12 months were unavailable for the remaining 2 children, seroconversion was evident in both between 6 and 12 months after birth. DNA (1 μg) isolated from each of the cord blood samples was analyzed by the nested PCR, with negative results for both the gag and pX regions[31]. The nested PCR was capable of amplifying a single molecule of HTLV-1 proviral DNA[30]. Since 1 μg of cellular DNA was equivalent to almost 2 x 10^6 cells, the tested cord blood samples contained no, or, if present, less than 0.0005%, HTLV-1-infected cells. This finding strongly argued against intrauterine infection in these children.

Evaluation of saliva as an alternative source for postnatal maternal HTLV-I transmission

Yamamoto et al.[32] evaluated saliva as an alternative source of postnatal transmission because of the following reasons. (i) Saliva contains a significant number of cells including lymphocytes. (ii) It can be transferred from mothers to children by kissing or sharing tableware. In particular, it is a Japanese custom for mothers to give food chewed by themselves to their children during the weaning period. They used PCR to detect and quantify the HTLV-I provirus in saliva samples of 18 carrier mothers and 10 HAM/TSP patients. The provirus was detected in 60 and 90%, respectively, of the samples, with estimated copy numbers in the range of 10-10^4/ml[32]. However, the saliva, regardless of the presence or absence of antibodies to the virus, showed a strong tendency to inhibit the cell-to-cell transmission of HTLV-I in vitro, as examined by syncytium inhibition assay. The natural inhibitory activity in saliva of a seronegative volunteer was heat-sensitive and ultrafiltration recovered most of the activity into the fraction of macromolecules with a molecular weight of more than 100 kilodaltons (Table 3)[32]. In addition to this natural activity, saliva of HTLV-I-infected individuals was shown to contain IgG molecules capable of neutralizing the syncytium formation[32]. These results strongly suggested that HTLV-I-infected cells in the carriers' saliva, which contains neutralizing antibodies in addition to the natural activity inhibiting cell-to-cell viral infection, barely transmit the virus. Moreover, the infected cell number of saliva potentially transferred from mothers to children are presumed to be much smaller than that of breast milk.

As described above, transfer of a huge number of infected cells, about 10^8 cells, through breast-feeding results in the transmission rate, as high as 20-30%. We thus concluded that transmission of HTLV-I through the saliva would be rare, if at all, although well-controlled epidemiological studies are needed to confirm the conclusion.

Table 3. Characteristics of HTLV-I inhibitory activity in saliva

Treatment	IC_{50} (log2)[a]	Relative inhibitory activity (%)
None	5.5	100
Heating, 56℃, 30 min	2.9	16
Centricon 100 (mol wt)[b]		
Filtrate (<100,000)	1.2	5
Retentate (>100,000)	>6.0	>141

a The concentration allowing 50% inhibition of the number of syncytia
b molecular weight (dalton)

Conclusion and perspective

Alternative pathways of maternal HTLV-I transmission other than via breast milk remain to be identified. Our studies did not support the possibilities of intrauterine transmission and infection via saliva. Among maternal pathways, perinatal transmission has yet to be evaluated. However, Sawada et al. reported the detection of HTLV-I proviral DNA in oral aspirates of newborns born to carrier mothers[33]. Perinatal infection of hepatitis B virus and HIV is well known. These two viruses transmit in cell-free manner, in contrast to the cell-to-cell infection of HTLV-I. Even so, the perinatal pathway should be evaluated as a likely candidate for alternative HTLV-I transmission.

At present, refraining from breast-feeding is the most effective measure to break the cycle of maternal HTLV-I transmission in endemic areas and to prevent the future development of ATL. The intervention in Nagasaki has revealed that about 85% of maternal HTLV-I transmission can be prevented by the refraining from breast-feeding by carrier mothers. If the intervention will be continued, the current carrier rate of 4% in Nagasaki is expected to decline to 0.4% in the next generation, and the annual incidence of ATL, 100, will be reduced to 10. I am reasonably confident that incidence of ATL in Nagasaki will decline to the national average level over the next few generation if the intervention program stays alive.

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