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Effect of Freeze-Thawing Breast Milk on Vertical HTLV-I Transmission from Seropositive Mothers to Children

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Breast feeding is known to be a major cause of vertical transmission of HTLV-I from mothers to her children. The infectiousness of HTLV-I in breast milk was reported to be lost during freezing and thawing processes. We therefore administered frozen-and-thawed breast milk of HTLV-I carriers to their babies. Among the 13 babies given the frozen-and-thawed breast milk (now 12 months of age), no infection has been found yet. This result suggests that freezing and thawing of breast milk is a promising method for the prevention of vertical HTLV-I infection to breast-fed babies.

Key words: HTLV-I — Vertical infection — Freeze-thawing process — Breast milk

Adult T-cell leukemia (ATL) is caused by human T lymphotropic virus type I (HTLV-I).[1-4] This virus is transmitted not only via blood transfusion[5] but also via familial contact (familial infection).[6-8] Familial HTLV-I infection includes vertical transmission from an HTLV-I seropositive mother to her children.[9] Breast feeding was reported to play a principal role in vertical transmission of HTLV-I.[10, 11] Some investigators have reported[10, 11] that vertical HTLV-I infection could be prevented almost completely by feeding artificial milk to babies. However, complete restraint of breast feeding involves some disadvantages for neonates in terms of gastroenteric infections, for example. Inactivation of HTLV-I infectiousness in the breast milk from HTLV-I carriers has been reported by Yamato *et al.*[12] and Ando *et al.*[13] In our recent study, we adopted the freezing-and-thawing process reported by Ando *et al.*[13] ($-20°$ for 12 h). After obtaining informed consent from each HTLV-I-carrying pregnant woman, we applied this method to breast milk. In addition, for the purpose of studying the mechanism by which this freeze-thawing process prevents HTLV-I infection, we observed the cells contained in frozen-and-thawed breast milk of HTLV-I carriers under an electron microscope.

The presence/absence of HTLV-I infection was assessed by the indirect immunofluorescence method reported by Hinuma *et al.*[1] Antibodies to HTLV-I were assessed by the use of MT-1. For detection of HTLV-I antigens, peripheral blood lymphocytes from babies were cultured for 4 weeks in RPMI-1640 supplemented with 20% fetal calf serum and 25% crude IL-2[14] in humidified 5% CO_2 air. After 4 weeks, they were washed with phosphate-buffered saline three times, smeared on a glass slide, and fixed with acetone at room temperature. The cells were subsequently treated with primary antibodies Gin-14[15] and F-10.[16] To explore the changes in the cells after freeze-thawing, the thawed breast milk was centrifuged at 200*g* for 10 min, and the precipitate was fixed in 2.5% glutaraldehyde and stained with osmium for electron microscopy.

In all babies born to HTLV-I carriers, antibodies to HTLV-I were detected in umbilical cord blood, although none showed the expression of HTLV-I antigens. The antibodies to HTLV-I detected in these babies gradually decreased with time. At the age of 9 months, no baby was HTLV-I antibody-positive. Of the 13 babies studied (12 months of age at present), none has shown the expression of HTLV-I antigens (Table I).

The changes observed in the cells contained in frozen-and-thawed breast milk consisted of destruction of the organelles and an amorphous change of the nucleus (Figs. 1 and 2). Thus, the cells contained in breast milk were found to lose their function in the course of freezing and thawing. Considering the known mechanism of intercellular HTLV-I infection,[17] this change of the cells supports the hypothesis that the freeze-thawing process of breast milk can prevent vertical HTLV-I infection. The absence of HTLV-I infection in the babies fed with the frozen-and-thawed breast milk indicates the practical value of this method in the sense that it allows HTLV-I carrying mothers to breast-feed their children, although indirectly.

The results of the present study provide evidence for the absence of HTLV-I infectiousness in frozen-and-

Table I. HTLV-I Antigen and Antibody Detection by Indirect Immunofluorescence in 13 Frozen-and-Thawed Breast Milk-fed Infants and 31 Non-treated Breast Milk-fed Infants Born to Seropositive Mothers

	Cord blood	Age (months)				
		1	3	6	9	12
Frozen-and-thawed milk						
HTLV-I Ag.[a]	0/13	0/13	0/13	0/13	0/13	0/13
HTLV-I Ab.[b]	13/13	13/13	10/13	6/13	0/13	0/13
Non-treated milk						
HTLV-I Ag.[a]	0/31	0/31	0/31	0/31	9/31	14/31
HTLV-I Ab.[b]	31/31	31/31	25/31	13/31	0/31	2/31

a) HTLV-I antigen-positive is defined as over 0.1% HTLV-I antigen-positive cell rate.
b) HTLV-I antibody-positive is defined as over ×5 titer.

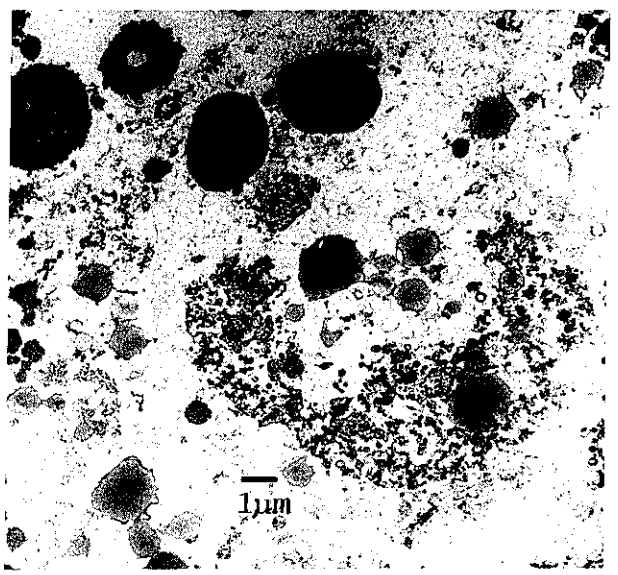

Fig. 1. Cells in frozen-and-thawed milk.

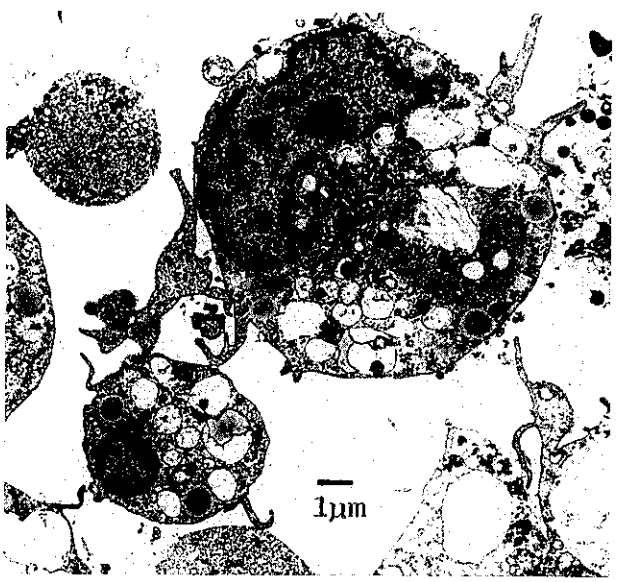

Fig. 2. Cells in unprocessed (fresh) breast milk.

thawed breast milk. However, the period of breast nursing in the present study was a little shorter than the ordinary period of breast feeding. At present, it is not clear to what extent the incidence of infection is affected by the duration of breast feeding. Therefore, it is necessary to observe the babies given frozen-and-thawed breast milk as to the presence/absence of HTLV-I infection over a longer period.

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