

CALL #: [http://0-www.interscience.wiley.com.library.uark.edu/jpages/...](http://0-www.interscience.wiley.com.library.uark.edu/jpages/)
LOCATION: **AFU :: Main Library :: [Japanese journal of cancer research (1988-2002) displays with Cancer science] Wiley Interscience.**

TYPE: Article CC:CCL
JOURNAL TITLE: Cancer science
USER JOURNAL TITLE: Cancer science
AFU CATALOG TITLE: Japanese journal of cancer research [electronic resource]
ARTICLE TITLE: Effect of Freeze-Thawing Breast Milk on Vertical HTLV-I Transmission from Seropositive Mothers to Children
ARTICLE AUTHOR: Ando, Yoshiya
VOLUME: 80
ISSUE: 5
MONTH:
YEAR: 1989
PAGES: 405-407
ISSN: 1347-9032
OCLC #: AFU OCLC #: 38911093
CROSS REFERENCE ID: 792479
VERIFIED:

BORROWER: **AZS :: Main Library**
PATRON: **Williams, Lyndsay**

PATRON ID:
PATRON ADDRESS:
PATRON PHONE:
PATRON FAX:
PATRON E-MAIL:
PATRON DEPT:
PATRON STATUS:
PATRON NOTES: google



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

Yoshiya Ando,¹ Kazuhiro Kakimoto,¹ Takuo Tanigawa,¹ Kazuo Furuki,¹ Kensuke Saito,¹ Shiro Nakano,¹ Hiratsugu Hashimoto,¹ Ikuko Moriyama,¹ Motohiko Ichijo¹ and Takenori Toyama²

¹Obstetrics and Gynecology, Nara Medical University, Shijo-cho 840, Kashihara, Nara 634 and ²Toyama Obstetrical and Gynecological Clinic, Uchidomari 127, Ginowan, Okinawa 901-22

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 transfusion⁵ 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-2¹⁴ in humidified 5% CO₂ 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-14¹⁵ and F-10.¹⁶ To explore the changes in the cells after freeze-thawing, the thawed breast milk was centrifuged at 200g 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 $\times 5$ titer.

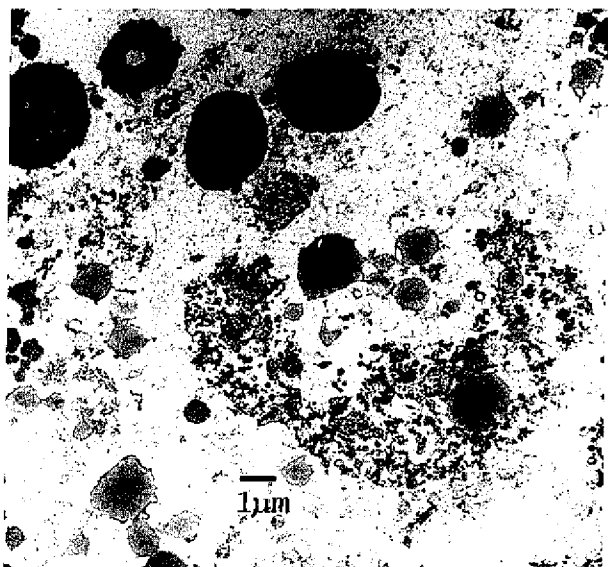


Fig. 1. Cells in frozen-and-thawed milk.

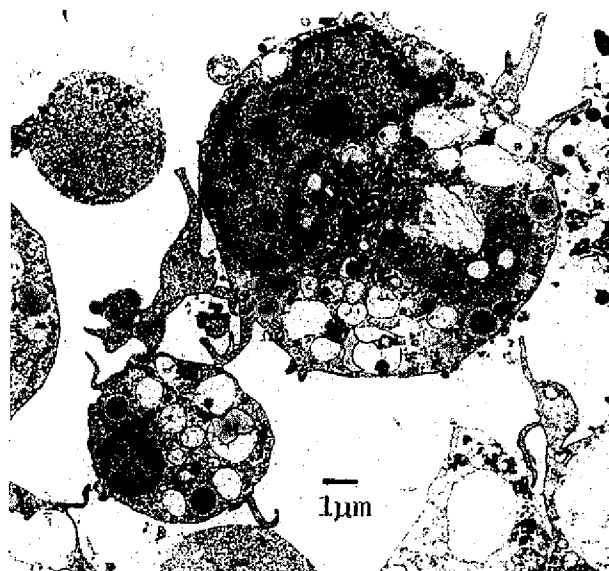


Fig. 2. Cells in unprocessed (fresh) breast milk.

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.

This work was supported in part by Grants-in-Aid from the Ministry of Health and Welfare, Japan.

(Received January 20, 1989/Accepted March 25, 1989)

REFERENCES

- 1) Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K., Shirakawa, S. and Miyoshi, I. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. USA*, **73**, 6476-6481 (1981).
- 2) Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagata, K. and Hinuma, Y. Type C virus particles in a cord T-cell line derived by co-cultivating normal cord leukocytes and human leukemic T-cells. *Nature*, **294**, 770-771 (1981).

- 3) Yoshida, M., Miyoshi, I. and Hinuma, Y. Isolating and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc. Natl. Acad. Sci. USA*, **79**, 2031-2035 (1982).
- 4) Kalyanaraman, V. S., Sarngadharan, M. G., Robert-Guroff, M., Miyoshi, I., Blayney, D., Golde, D. and Gallo, R. C. A new subtype of human T-cell variant of hairy cell leukemia. *Science*, **218**, 571-573 (1982).
- 5) Okochi, K., Sato, H. and Hinuma, Y. A retrospective study on transmission of adult T-cell leukemia virus by blood transfusion: seroconversion in recipients. *Vox Sang.*, **46**, 245-253 (1984).
- 6) Miyoshi, I., Taguchi, H., Fujishita, M., Niiya, K., Kitagawa, T., Ohtsuki, Y. and Akagi, T. Asymptomatic type C virus carriers in the family of an adult T-cell leukemia patient. *Gann*, **73**, 339-340 (1982).
- 7) Tajima, K., Tominaga, S., Suchi, T., Kawagoe, T., Komoda, H., Hinuma, Y., Oda, T. and Fujita, K. Epidemiological analysis of the distribution of antibody to adult T-cell leukemia-virus-associated antigen: possible horizontal transmission of adult T-cell leukemia virus. *Gann*, **73**, 893-901 (1982).
- 8) Kondo, T., Nonaka, H., Miyamoto, N., Yoshida, R., Matsue, Y., Ohguchi, Y., Inoue, H., Komoda, H., Hinuma, Y. and Hanaoka, M. Incidence of adult T-cell leukemia-lymphoma and its familial clustering. *Int. J. Cancer*, **35**, 749-751 (1985).
- 9) Nakano, S., Ando, Y., Saito, K., Moriyama, I., Ichijo, M., Toyama, T., Sugamura, K., Imai, J. and Hinuma, Y. Primary infection of Japanese infants with adult T-cell leukemia-associated retrovirus (ATLV): evidence for viral transmission from mothers to children. *J. Infect.*, **12**, 205-212 (1985).
- 10) Hino, S., Sugiyama, H., Doi, H., Ishimaru, T., Yamabe, T., Tsuji, Y. and Miyamoto, T. Breaking the cycle of HTLV-I transmission via carrier mother's milk. *Lancet*, **II**, 158-159 (1987).
- 11) Ando, Y., Nakano, S., Saito, K., Shimamoto, I., Ichijo, M., Toyama, T. and Hinuma, Y. Transmission of adult T-cell leukemia retrovirus (HTLV-I) from mother to child: comparison of bottle- with breast-fed babies. *Jpn. J. Cancer Res.*, **78**, 322-324 (1987).
- 12) Yamato, K., Taguchi, H., Yoshimoto, S., Fujishita, M., Yamashita, M., Ohtsuki, Y., Hoshino, H. and Miyoshi, I. Inactivation of lymphocyte-transforming activity of human T-cell leukemia virus type I by heat. *Jpn. J. Cancer Res.*, **77**, 13-15 (1986).
- 13) Ando, Y., Nakano, S., Saito, K., Shimamoto, I., Ichijo, M., Toyama, T. and Hinuma, Y. Prevention of HTLV-I transmission through the breast milk by a freeze-thawing process. *Jpn. J. Cancer Res.*, **77**, 974-977 (1986).
- 14) Tanaka, Y., Sugamura, K. and Hinuma, Y. T-cell growth factor from human splenic cell cultures: conditions for its production, and its utilization for maintenance of cytotoxic T cell lines. *Microbiol. Immunol.*, **25**, 341-344 (1981).
- 15) Tanaka, Y., Koyanagi, Y., Chosa, T., Yamamoto, N. and Hinuma, Y. Monoclonal antibody reactive with both p28 and p19 of adult T-cell leukemia virus-specific polypeptides. *Gann*, **74**, 327-330 (1983).
- 16) Sugamura, K., Fujii, M., Ueda, S. and Hinuma, Y. Identification of a glycoprotein, gp21 of adult T-cell leukemia virus by monoclonal antibody. *J. Immunol.*, **132**, 9180-9184 (1984).
- 17) Chosa, T., Yamamoto, N., Tanaka, Y., Koyanagi, Y. and Hinuma, Y. Infectivity dissociated from transforming activity in a human retrovirus, adult T-cell leukemia virus. *Gann*, **73**, 844-847 (1982).