

Human Colostrum and Breast Milk Contain High Levels of TNF-Related Apoptosis-Inducing Ligand (TRAIL)

Journal of Human Lactation
XX(X) 1–3
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DOI: 10.1177/0890334412441071
<http://jhl.sagepub.com>


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Abstract

Background: TNF-related apoptosis inducing ligand (TRAIL) is a pleiotropic cytokine, which plays a key role in the immune system as well as in controlling the balance of apoptosis and proliferation in various organs and tissues.

Objective: To investigate the presence and levels of soluble TRAIL in human colostrum and milk.

Methods: The levels of soluble human TRAIL were measured in human colostrum (day 2 after delivery) and breast milk (day 5 after delivery). The presence of TRAIL was also measured in infant formula.

Results: Levels of soluble TRAIL in the colostrum and mature human milk were, respectively, at least 400 and 100 fold higher than those detected in human serum. No TRAIL was detected in formula.

Conclusion: Human soluble TRAIL is present at extremely high levels in human colostrum and human milk and might have a significant role in mediating the anti-cancer activity of human milk.

Keywords

trail, colostrum, breast milk, immune regulation, anti-cancer activity

Well Established

Apoptosis (programmed cell death) is a major mechanism that protects us against cancer. TNF-related apoptosis inducing ligand (TRAIL) is an immunoactive substance that plays a key role in controlling apoptosis and cell proliferation in various organs and tissues.

Newly Expressed

We found extremely high levels of human soluble TRAIL in human colostrum and breast milk. TRAIL might play an important role in the anticancer activity of human milk.

Introduction

TRAIL is a member of the tumor necrosis factor (TNF) superfamily of cytokines, which exists as either a transmembrane or soluble protein.¹ The best characterized activity of TRAIL is to kill cancer cells both in vitro and in vivo, although this is a pleiotropic cytokine with important functions in regulating immune and inflammatory responses.¹ Besides being expressed by leukocytes, significant levels of TRAIL protein have been detected in many human tissues by immunohistochemistry or flow cytometry, including normal epithelia, such as corneal and conjunctival,² intestinal,³⁻⁵ and breast⁶ epithelia. In spite of these findings, little to nothing is known on the ability of different epithelia to release soluble TRAIL. On this basis, we have comparatively investigated the levels of soluble TRAIL in samples of human colostrum,

breast milk, serum, and infant formulas commonly given to infants as an alternative to breast milk.

Methods

The study was carried out at the regular nursery of the Institute for Maternal and Child Health “Burlo Garofolo” in Trieste, Italy. Mothers with no eclampsia, infection, or fever who delivered healthy newborns at term with a 5-minute Apgar score ≥ 7 and with no major congenital malformations were considered eligible to be enrolled in the study. Only 1 day per week, women were enrolled within the first 24 hours after delivery by specialized medical doctors assigned to the study during a period of 8 months (February

Received for review January 13, 2012; revised manuscript accepted for publication February 11, 2012.

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Table. Main Characteristics of the Mother and Baby Dyads from Which TRAIL Concentrations Were Determined in Colostrum (N = 55)

Characteristics	No., (average [SD] or median [IQR]) ^a
Maternal age at delivery (y)	34.5 (31 - 37)
Parity	
1	21
2	27
≥ 3	7
Mode of delivery	
Vaginal	40
Elective cesarean section	10
Urgent cesarean section	5
Preeclampsia	
No	53
Yes	2
Premature rupture of membranes	
No	38
Yes	17
Maternal autoimmune diseases	
No	53
Yes	2
Gestational wk at delivery	39.1 (1.2)
Newborn birth weight (g)	3377 (415)
APGAR at 1 minute	
8	53
9	2
APGAR at 5 minutes	
9	53
10	2

Abbreviations: IQR, inter quartile range, SD, standard deviation.

^aFor continuous variables not distributed normally, medians and interquartile ranges were calculated.

15-October 15, 2011), thus not all eligible women were approached for recruitment. Women were asked to sign an informed consent form. With the help of a nurse, women expressed 1 mL of colostrum by hand or/and by electric pump, at 24-48 hours after delivery. In case of a longer stay in the hospital because of cesarean section (CS), 1 mL of breast milk was also collected at 72-120 hours after delivery. We recorded data on maternal preeclampsia and autoimmune diseases (before and during the last pregnancy); maternal age at delivery; parity; type of birth; time of rupture of the membranes; birth weight and gestational age of the baby; and Apgar score (Table). Human serum samples (n = 40) were obtained from the blood bank of the University Hospital of Ferrara from healthy women.

All biological samples and 7 types of ready-to-feed (ie, liquid) infant formulas from 5 different brands were aliquoted, stored at -80°C, and thawed only once before the assays for TRAIL measurement by an enzyme-linked immunosorbent assay (ELISA). Analyses were performed using

specific, commercially available ELISA kits purchased from R&D Systems (Minneapolis, MN), performed as previously described by our research group.⁷

After verifying that TRAIL values did not distribute normally, we performed a nonparametric Mann-Whitney test to verify whether there was a significant difference in TRAIL values of the colostrum of women who had a vaginal versus cesarean delivery. To verify whether there was a difference in TRAIL values between colostrum and breast milk at 5 days after delivery, we performed a nonparametric signed-rank Wilcoxon test for matched pairs.

Results

A descriptive analysis on the 55 mother and baby dyads for which TRAIL values were measured in the colostrum is reported in the Table. For 10 of these 55 women, we also had TRAIL values in breast milk at day 4 or 5 after delivery, whereas for 7 women, we had values in breast milk only at day 4 or 5 after delivery.

The median TRAIL value measured in the 55 colostrum samples was 19.87 ng/mL (range, 1.50-298.16 ng/mL; Figure). We found no significant difference in colostrum TRAIL values between vaginal and cesarean section deliveries ($P = .21$). The median TRAIL value in the breast milk was 9.57 pg/mL (Figure). In the paired samples, according to the Wilcoxon nonparametric signed rank test, there was a significant difference in TRAIL concentrations between the samples of colostrum and samples of breast milk ($P = .007$), with median TRAIL values in breast milk about 4-fold lower than in colostrum (7.15 vs 30.30 ng/mL). It is particularly noteworthy that the levels of TRAIL in both colostrum and breast milk samples were much higher ($P < .001$) than those found in the serum samples of a group of age-matched healthy women, characterized by mean and median serum TRAIL values of 64 and 67 pg/mL, respectively (Figure). In all samples of liquid infant formulas analyzed, TRAIL was not detectable (data not shown).

Discussion

To our knowledge, this is the first time that TRAIL has been measured in colostrum and human breast milk. This study has revealed much higher TRAIL concentrations in colostrum and breast milk compared to the levels of circulating serum TRAIL. Although previous studies have shown that breast cells express TRAIL,⁶ it was unexpected that the mean level of soluble TRAIL detected in colostrum reached 48.6 ng/mL, approximately 2-fold higher than the level previously reported in the conjunctival sac fluid,² approximately 40-fold greater than the level measured in human saliva,² and > 400-fold greater than the level found in human serum. Although we observed a significant decline in levels of TRAIL in human breast milk measured at day 4 or 5, these concentrations were still much higher

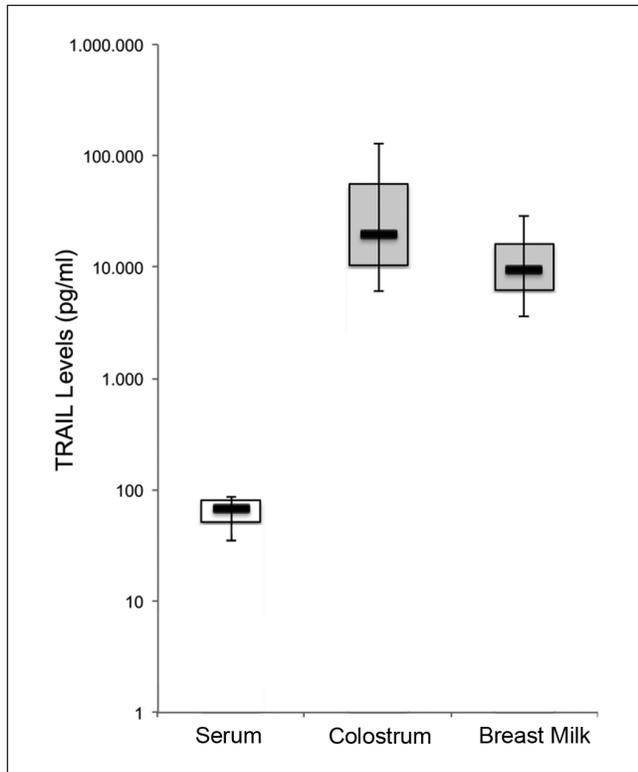


Figure. Comparative analysis of soluble TRAIL concentrations measured in samples of human colostrum, breast milk, and serum. Levels of TRAIL were determined by ELISA in samples of human colostrum ($n = 55$), breast milk ($n = 17$), and serum ($n = 40$). Horizontal bars are median, upper and lower edges of box are 75th and 25th percentiles, lines extending from box are 10th and 90th percentiles. $P = .007$ for comparison of 10 paired samples of TRAIL values in colostrum versus breast milk; $P < .001$ for comparison of unpaired samples of TRAIL values in breast milk versus human serum; $P = .003$ for comparison of unpaired samples of TRAIL values in colostrum versus human serum.

than those usually found in human serum/plasma and were in the range of concentrations that are able to kill cancer cells.¹ Conversely, TRAIL was not detected in ready-to-feed infant formulas.

It is well known that human milk is more than simple food for infants, representing a complex biological liquid containing many immunoactive agents. In the context of our study, the important role of breastfeeding in the prevention of certain childhood cancers, such as lymphoblastic leukemia, Hodgkin's disease, and neuroblastoma, has been previously demonstrated,⁸ and the presence in human milk of substances with antitumor activity, such as human alpha-lactalbumin made lethal to tumor cells (HAMLET), has been reported.⁹ However, in consideration of the strong anticancer activity of TRAIL, which is currently used as a recombinant protein in many clinical trials against a variety of human cancers,¹⁰ endogenous soluble TRAIL represents a strong candidate to explain the overall biological effect of

breastfeeding against cancer. In this respect, it has recently been shown that certain *Lactobacillus* strains induce TRAIL production and facilitate natural killer activity against cancer cells.⁵

Thus, our hypothesis is that high concentrations of soluble TRAIL present in human colostrum and breast milk might play a key role in mediating the anticancer activity of human milk.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: the study was approved by the Research Commission of the IRCCS "Burlo Garofolo," Trieste. The authors have no financial relationships relevant to this article to disclose.

References

- Di Pietro R, Zauli G. Emerging non-apoptotic functions of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L. *J Cell Physiol.* 2004;201:331-340.
- Secchiero P, Lamberti G, Corallini F, et al. Conjunctival sac fluid contains elevated levels of soluble TRAIL: implications for the anti-tumoral surveillance of the anterior surface of the eye. *J Cell Physiol.* 2009;218:199-204.
- Rimondi E, Secchiero P, Quaroni A, Zerbinati C, Capitani S, Zauli G. Involvement of TRAIL/TRAIL-receptors in human intestinal cell differentiation. *J Cell Physiol.* 2006;206:647-654.
- Brost S, Koschny R, Sykora J, et al. Differential expression of the TRAIL/TRAIL-receptor system in patients with inflammatory bowel disease. *Pathol Res Pract.* 2010;206:43-50.
- Horinaka M, Yoshida T, Kishi A, et al. *Lactobacillus* strains induce TRAIL production and facilitate natural killer activity against cancer cells. *FEBS Lett.* 2010;584:577-582.
- Rachner TD, Singh SK, Schoppet M, et al. Zoledronic acid induces apoptosis and changes the TRAIL/OPG ratio in breast cancer cells. *Cancer Lett.* 2010;287:109-116.
- Campioni D, Secchiero P, Corallini F, et al. Evidence for a role of TNF-related apoptosis-inducing ligand (TRAIL) in the anemia of myelodysplastic syndromes. *Am J Pathol.* 2005;166:557-563.
- Martin RM, Gunnell D, Owen CG, Smith GD. Breast-feeding and childhood cancer: a systematic review with metaanalysis. *Int J Cancer.* 2005;117:1020-1031.
- Mossberg AK, Hun MK, Morozova-Roche LA, Svanborg C. Structure and function of human alpha-lactalbumin made lethal to tumor cells (HAMLET)-type complexes. *FEBS J.* 2010;277:4614-4625.
- Mahalingam D, Oldenhuis CN, Szegezdi E, et al. Targeting trail towards the clinic. *Curr Drug Targets.* 2011;12:2079-2090.