

Journal of Human Lactation

<http://jhl.sagepub.com/>

Characterization of Immunocompetent Cells in Human Milk of Han Chinese

Ying-Ying Jin, Wei Zhao, Rui-Ming Cao, Xi Wang, Sheng-Mei Wu and Tong-Xin Chen

J Hum Lact published online 10 March 2011

DOI: 10.1177/0890334410392041

The online version of this article can be found at:

<http://jhl.sagepub.com/content/early/2011/03/10/0890334410392041>

Published by:



<http://www.sagepublications.com>

On behalf of:



[International Lactation Consultant Association](#)

Additional services and information for *Journal of Human Lactation* can be found at:

Email Alerts: <http://jhl.sagepub.com/cgi/alerts>

Subscriptions: <http://jhl.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

Characterization of Immunocompetent Cells in Human Milk of Han Chinese

Ying-Ying Jin, MD, Wei Zhao, MD, PhD, Rui-Ming Cao, MD, Xi Wang, MD, Sheng-Mei Wu, MD, and Tong-Xin Chen, MD, PhD

Abstract

Immunocompetent cells in colostrum and mature breast milk (42 days after delivery) of 64 Chinese females of Han ethnic background were characterized. The total cell numbers in colostrum and mature breast milk were $593 \pm 205 \times 10^6/\text{L}$ and $1.74 \pm 0.97 \times 10^6/\text{L}$, respectively. NK cell is the predominant lymphocyte subtype (12.69%). T cells in colostrum express CD40L and CD25 without stimulation, indicating memory T cells in nature. The CD45RA⁺CD45RO⁺ subsets of CD4⁺ T cells are present in both colostrum and mature human milk. The percentage of NK cells and the absolute number of CD4⁺CD45RO⁺ T cells in colostrum of atopic mothers were significantly lower than those of mothers without atopy ($P = .006$ and $.026$, respectively). The absolute numbers of CD4⁺ T lymphocytes and their expression of CD40L were significantly reduced in breast milk of mothers who had undergone cesarean section versus vaginal delivery ($P < .05$), indicating that atopy and cesarean delivery are associated with the phenotypes of lymphocytes in colostrum.

Keywords: colostrum, mature milk, lymphocyte, flow cytometry, breast feeding

Human breast milk provides a communication mechanism between the maternal and infant immune system by delivering immunocompetent factors. It is well accepted that exclusive breastfeeding is the optimal feeding choice for the first 6 months of life. Upon birth, neonates are directly exposed to microorganisms, foreign proteins,

and chemicals in their environment. The immune system of newborn infants is undergoing rapid maturation. Human breast milk, particularly colostrum, can provide not only nutritional needs for growing but also immunologic components such as polyunsaturated fatty acids, IgG, IgM, secretory IgA, soluble CD14, lactoferrin, and cytokines, which can facilitate the development of infant immune system, immunotolerance, and appropriate inflammatory response. Of note is that the protective effects seem sustained beyond the time of lactation and may even last through adulthood.¹ It remains to be determined how these immunologic components in breast milk with half-lives of hours to a few days can perpetuate their long-lasting protective effects. Understanding such mechanisms may shed light on the development of neonatal immune system.

Numerous factors have been discovered in human milk, with some variation in their quantity among different ethnic backgrounds. Kneebone et al² showed that fatty acid composition of breast milk from 3 racial groups (Malay, Chinese, Indian) from Penang, Malaysia, was different, with less saturated fatty acid and higher linoleic acid in Chinese than in Malay and Indian mothers because of the distinct cultural differences in dietary intake.² De Amici et al³ reported that racial/ethnic factors are predictive of the onset of lactation and the volume of breast milk. However, it is undetermined whether such a difference is present in immunocompetent cells in human milk.

Received for review April 15, 2010; revised manuscript accepted for publication October 26, 2010.

No competing interests.

Ying-Ying Jin, MD, is with the Department of Pediatrics, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. **Wei Zhao**, MD, PhD, is with the Division of Allergy and Immunology, Department of Pediatrics, Virginia Commonwealth University. **Rui-Ming Cao**, MD, is with the Department of Immunology, Shanghai Institute for Pediatric Research, Shanghai Jiao Tong University School of Medicine. **Xi Wang**, MD, is with the Department of Immunology, Shanghai Institute for Pediatric Research, Shanghai Jiao Tong University School of Medicine. **Sheng-Mei Wu**, MD, is with Beingmate Research Center for Human Breast Milk, Shanghai Institute for Pediatric Research, Shanghai Jiao Tong University School of Medicine. **Tong-Xin Chen**, MD, PhD, is with the Department of Pediatrics, Xinhua Hospital, and the Department of Immunology, Shanghai Institute for Pediatric Research, Shanghai Jiao Tong University School of Medicine.

The study was funded by Beingmate Group Co., Ltd.

Address correspondence to Prof. Tong-Xin Chen, Department of Immunology, Shanghai Institute for Pediatric Research, Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China; e-mail: tongxinc@yahoo.com.

J Hum Lact XX(X), XXXX

DOI: 10.1177/0890334410392041

© Copyright 2011 International Lactation Consultant Association

The distribution of immunocompetent cells in human milk has been reported in Caucasian mothers. Total leukocyte numbers are $4 \times 10^9/L$ and 10^8 to 10^9 in colostrum and mature breast milk, with macrophages (55%-60%) and neutrophils (30%-40%) but not lymphocytes (5%-10%) being the dominating cell types.⁴ T cells account for more than 80% of the lymphocytes in human milk; most of them are CD8 positive.⁵ They may be derived from the maternal mucosal immune system. In the present study, the immunocompetent cells in human milk of Han Chinese in Shanghai are characterized and the associations of delivery mode and atopy with phenotypes of lymphocytes in colostrum are explored.

Methods

Milk Collection

Colostrum samples were obtained within 3 days of parturition from 64 healthy mothers and mature breast milk was obtained on day 42 from the same mothers of term infants between March 2008 and May 2008 in the Department of Gynecology and Obstetrics of Xinhua Hospital, Shanghai Jiao Tong University School of Medicine. All the mothers recruited belonged to middle/higher social classes from an urban environment, practiced exclusive breastfeeding, and had no history of infection since 4 weeks before parturition. Written informed consent was obtained from these mothers. The study was approved by the Human Ethics Committee of Xinhua Hospital.

Medical records were reviewed; delivery mode, atopy status, and all variables of the mothers are described in Table 1. Mothers with history of atopic dermatitis, allergic rhinitis, drug allergy, or asthma were considered to have allergy. Breast milk was collected using a manual breast pump after local areas were washed. The collection took place in the morning before the second feeding of the day. Two milliliters of breast milk was collected and stored in sterile 10-mL tubes.

Separation and Purification of Milk Leukocytes

The cellular components of breast milk were isolated as described by Järvinen and Suomalainen.⁶ Briefly, the breast milk was centrifuged at 400g at room temperature for 15 minutes. Fat containing supernatant was removed. Leukocytes were collected and washed 3 times for 15 minutes each in complete RPMI-1640 medium containing 5% fetal bovine serum and antibiotics (penicillin and streptomycin). After washing, the cells were resuspended in the same medium and were counted in a hemocytometer. Cell viability was determined by trypan blue exclusion and was greater than 98%.

Table 1. General Clinical Characteristics of Parents and Neonates

Object	Features
<i>Mothers (n = 64)</i>	
Birthplace	Shanghai
Nationality	Han Chinese
Age, y	28.91 ± 3.01
Parity	
Primipara	60
Pluripara	4
Delivery	
Vaginal	22
Cesarean	42
Smoking history	
(+) passive	26
(-) passive	38
Allergy history	
Positive	22 ^a
Negative	42
<i>Fathers (n = 64)</i>	
Allergy history	
Positive	12 ^b
Negative	52
Occupations	42 clerks, 4 nurses, 4 salespersons, 2 attendants, 12 unemployed
<i>Neonate (n = 64)</i>	
Gestation age, wk	39.09 ± 0.91
Birth weight, g	3522.26 ± 457.56
Apgar score	10
Sex	34 females, 30 males

^a4 atopic dermatitis, 6 allergic rhinitis, 9 drug allergy, 2 food allergy, 1 asthma; 10 with vaginal delivery and 12 with cesarean delivery.

^b2 atopic dermatitis, 2 allergic rhinitis, 6 drug allergy, 2 food allergy.

Microscopic Examination of Human Milk Leukocytes

The human milk leukocytes were cytopun and stained with May-Grunwald-Giemsa solution. Cell types such as macrophages, monocytes, lymphocytes, and neutrophils were determined by examination under light microscope.

Flow Cytometry Study of Human Milk Leukocytes

Flow cytometry was used to determine the expression of different surface markers of human milk leukocytes. Cells were suspended in complete RPMI-1640 and incubated with different monoclonal antibodies (Becton-Dickinson Immunocytometry Systems, Mountain View, California) at room temperature for 15 minutes. Cell subtypes were classified based on the pattern of their surface marker expression. CD3-PerCP⁺CD4-FITC⁺ represents helper T cells; CD3-PerCP⁺CD8-PE⁺, cytotoxic T cells; CD3-FITC⁺CD19-PerCP⁺, B cells; CD3-FITC⁺CD16+56-PE⁺, NK cells; CD14-FITC⁺, monocytes; CD4-PerCP⁺CD45RA-FITC⁺, naive T cells; CD4-PerCP⁺CD45RO-PE⁺, memory T cells; CD4-PerCP⁺CD25-PE⁺ or CD4-PerCP⁺CD40L-PE⁺, activated

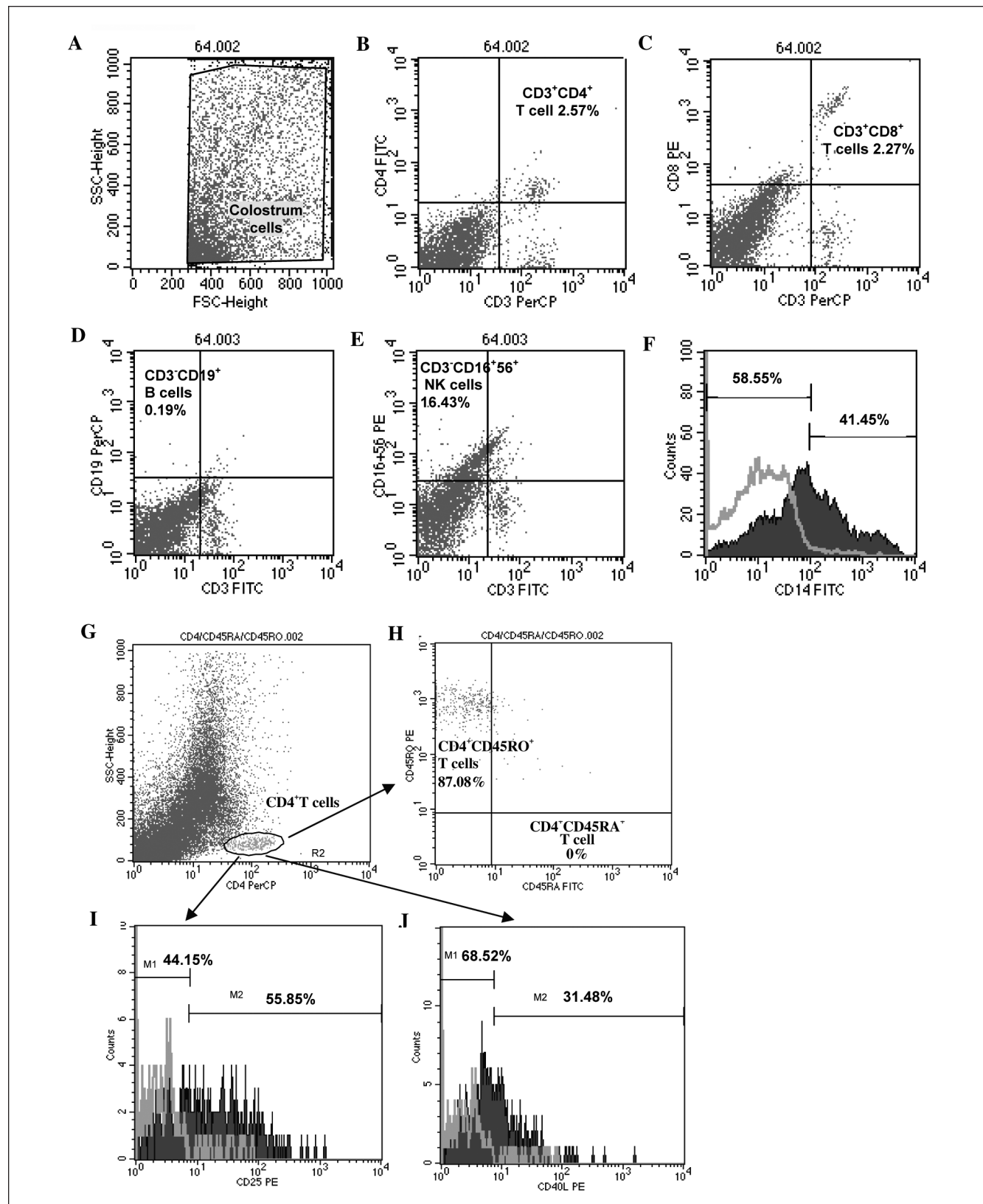


Figure 1. Flow cytometry study of human milk leukocytes. (A) Forward- and side-scatter of human milk leukocytes. (B) CD3, CD4 staining. (C) CD8 staining. (D) CD19 staining. (E) CD16/56 staining. (F) CD14 staining. (G) Gating for CD4 positive T leukocytes in human milk. (H, I, J) Expression of CD45RO/RA, CD25 and CD40L of CD4⁺ T leukocytes.

Table 2. Distribution of T Lymphocytes in Colostrum and Mature Milk (n = 64)

		<i>T lymphocytes (%)</i>						
		<i>CD3⁺</i>	<i>CD3⁺CD4⁺</i>	<i>CD3⁺CD8⁺</i>	<i>CD4⁺CD45RA⁺</i>	<i>CD4⁺CD45RO⁺</i>	<i>CD4⁺CD40L⁺</i>	<i>CD4⁺CD25⁺</i>
Colostrum, × 10 ⁶ /L	Median	18.51 (3.63)	3.91 (0.65)	5.96 (1.07)	0.00 (0.00)	3.44 (85.07)	1.09 (27.86)	1.68 (56.83)
	95% CI	16.80-28.16 (3.02-4.50)	3.54-7.23 (0.63-1.20)	6.39-10.96 (1.13-1.79)	0.00-0.10 (0.13-1.45)	3.03-6.61 (81.11-87.86)	1.24-3.30 (24.84-40.11)	1.95-4.50 (49.02-59.34)
Mature milk, × 10 ⁴ /L	Median	5.77 (4.06)	1.76 (0.96)	1.50 (0.77)	0.00 (0.00)	1.26 (73.33)	0.61 (36.96)	0.54 (34.85)
	95% CI	5.68-9.12 (3.66-6.22)	1.82-3.31 (1.15-2.20)	1.33-2.77 (0.81-2.05)	0.01-0.02 (0.22-0.94)	1.28-2.60 (64.86-77.29)	0.62-1.22 (31.62-42.10)	0.59-1.32 (31.78-40.05)

Abbreviations: CI, confidence interval.

T cells. Data from 100 000 events were acquired by FACS Calibur (Becton-Dickinson, Franklin Lake, New Jersey). Appropriate threshold was set to exclude cell debris and gate was set to include all leukocytes. Data were analyzed by CELL-QUEST software. Figure 1 shows the gate strategy, which was representative of flow cytometry data of human milk leukocytes. The same instrument setting was used for analyzing colostrum and mature milk samples.

Statistical Analysis

Statistical analysis was performed with SPSS 14.0. Descriptive data were expressed as median and 95% confidence interval (CI). The medians were used for data that were not normally distributed. The differences between 2 groups (vaginal vs cesarean delivery and atopy vs nonatopy) were evaluated by Mann-Whitney *U* test. *P* < .05 was considered significant.

Results

General Clinical Features of Mothers and Neonates

All 64 mothers in this study are Shanghai natives with Han nationality. Their ages were between 25 and 35 years (mean age, 28.91 ± 3.01 years). All but 4 were primiparas. Twenty-two had spontaneous vaginal delivery and 42 underwent elective cesarean delivery. Other demographic parameters such as parental smoking history, allergy history, occupations, and the detailed history of neonates were collected and are shown in Table 1.

General Features of Colostrum and Mature Milk

The total cell counts of colostrum and mature milk were (593 ± 205) × 10⁶/L and (1.74 ± 0.97) × 10⁶/L, respectively. After May-Grunwald-Giemsa stain, cells were readily distinguished among lymphocytes, monocytes, and neutrophilic granulocytes.

Distribution of Leukocytes in Human Milk

For colostrum, the total cell number was (593 ± 205) × 10⁶/L. The percentages of CD3⁺, CD19⁺, CD16⁺/CD56⁺, and CD14⁺ monocytes (95% CI) in colostrum were 3.63% (3.02%-4.50%), 0.58% (0.45%-0.99%), 12.69% (10.69%-18.08%), and 25.02% (25.88%-37.92%), respectively. The absolute numbers of the above cell types (95% CI) were 18.51 × 10⁶/L (16.80-28.16 × 10⁶/L), 2.31 × 10⁶/L (2.31-6.09 × 10⁶/L), 66.69 × 10⁶/L (58.37-107.39 × 10⁶/L), and 157.96 × 10⁶/L (144.35-216.35 × 10⁶/L), respectively (Figure 1). CD8⁺ cells were slightly higher than CD4⁺ cells in CD3⁺ lymphocyte; the mean ratio of CD4/CD8 was 0.59 (0.32-1.09). Subsets of such CD4⁺ cells in human colostrum were further characterized by examining their surface marker expression. The results, as shown in Table 2, revealed that CD45RO⁺ T cells (memory T cell) are the major subset (85.07%) of CD3⁺CD4⁺ T lymphocytes. In contrast, CD45RA⁺ T cells (naive T cell) were hardly detectable. The CD4⁺ T lymphocytes in colostrum express CD40L and CD25 (IL-2Rα) without any stimulation (Table 2).

For mature milk, the total cell number (1.74 ± 0.97 × 10⁶/L) was significantly lower than colostrum (593 ± 205 × 10⁶/L). All subsets of cells in mature milk were significantly lower than colostrum. CD3⁺ T lymphocytes were 309-fold lower in mature milk, whereas CD16⁺/CD56⁺ T lymphocytes were 953-fold lower. CD14⁺ monocytes were the main cell subtype in colostrum as well as mature breast milk.

Existence of CD45RA⁺CD45RO⁺ Cells in Both Colostrum and Mature Human Milk

The numbers (95% CI) of CD45RA⁺CD45RO⁺ in colostrum and mature human milk were 35.50 (37.03-84.11) × 10⁴/L and 0.18 (0.14-0.33) × 10⁴/L, respectively. Those cells account for 12.24% ± 7.99% (colostrum) and 9.52% ± 7.26% (mature milk) of CD4⁺ T cells.

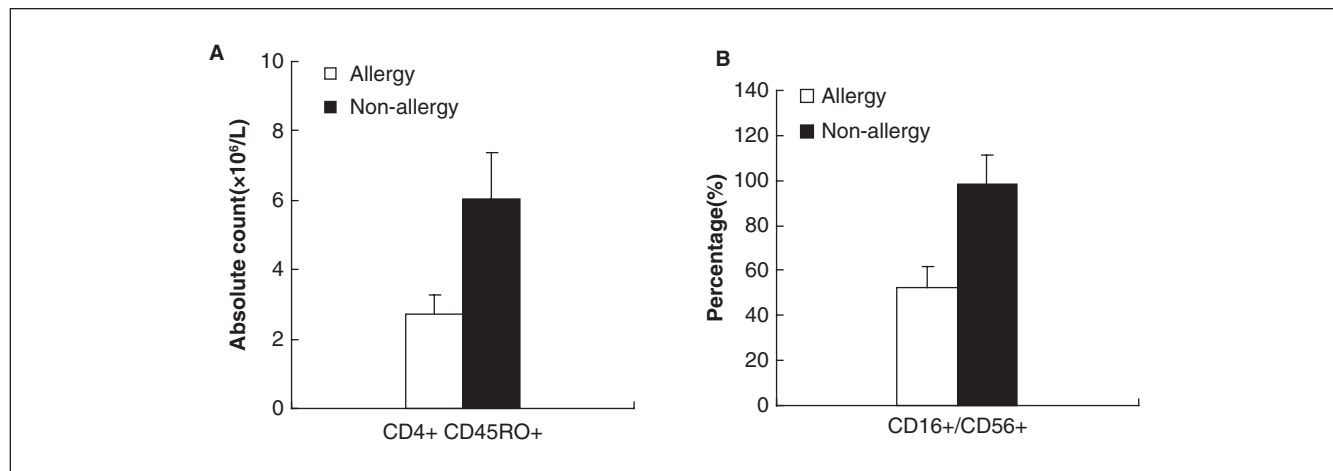


Figure 2. The association of atopy on phenotypes with lymphocytes in colostrum. (A) Data expressed as mean $\pm$ SD of mothers with atopy ($n = 22$) and without atopy ($n = 42$), $P = .026$. (B) Comparison of CD16⁺/CD56⁺ T cells (percentage) between the 2 groups, $P = .006$. Mothers with history of atopic dermatitis, allergic rhinitis, drug allergy, or asthma were considered to have allergy.

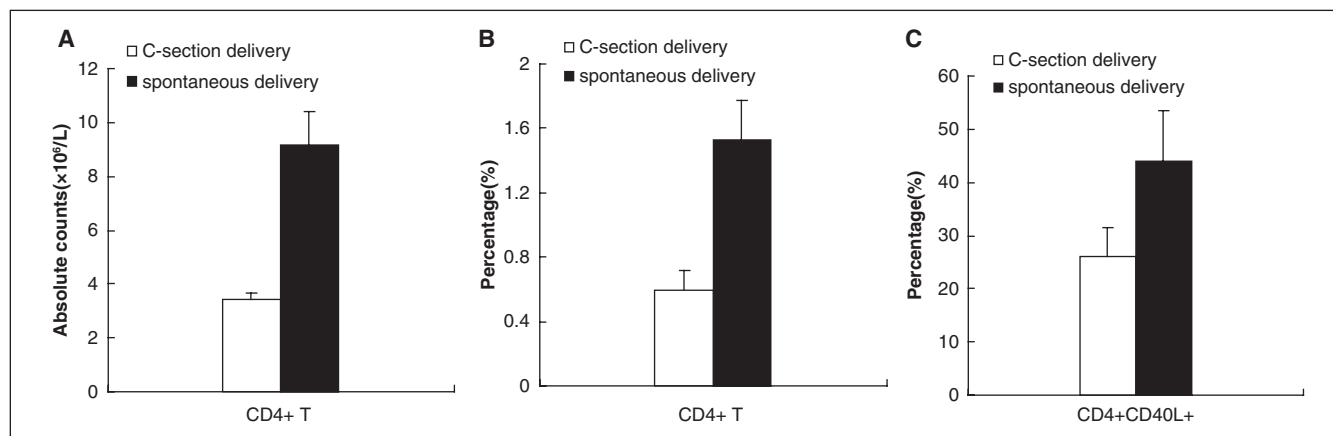


Figure 3. The association of labor presentation with phenotypes of lymphocytes in colostrum. Percentages of cell subtypes were determined by flow cytometry. (A) Comparison of CD4⁺ T cells (absolute count) between mothers with cesarean delivery ($n = 22$) and spontaneous delivery ($n = 42$), $P = .012$. (B) Comparison of CD4⁺ T cells (percentage) between groups, $P = .006$. (C) Comparison of activated T cells (percentage) between groups, $P = .021$.

Association of Atopy and Cesarean Delivery With Phenotypes of Lymphocytes in Human Milk

To examine whether atopy and cesarean delivery were associated with lymphocyte populations in human milk, we analyzed the number of T-cell subsets in mothers with history of atopy and mothers with cesarean delivery. For colostrum, as demonstrated in Figure 2, both the percentage of NK cells and the CD4⁺CD45RO⁺ T cells in colostrum of atopic mothers were significantly lower than those of nonatopic mothers ($P = .006$, $P = .026$, respectively). The absolute number and the percentage of CD4⁺CD25⁺ T cells did not differ between the atopic

group and the nonatopic group ($P = .420$, $P = .333$, respectively). The number of CD4⁺ cells and their expression of CD40L were significantly reduced in colostrum of mothers who had undergone cesarean delivery (Figure 3, $P < .05$). However, the cellular composition of human colostrum was comparable between mothers with or without a history of passive smoking (data not shown). For mature human milk, the expression of CD40L on CD4⁺ T cells was significantly reduced in atopic mothers, and the difference between atopic mothers and nonatopic mothers was significant ($0.41 \pm 0.12 \times 10^4/L$ vs $1.10 \pm 0.68 \times 10^4/L$, respectively, $P = .002$).

Discussion

The current study explored the phenotype and subsets of immunocompetent cells in human milk of Han Chinese in Shanghai and further analyzed the association of atopy and cesarean delivery with phenotypes of lymphocytes in breast milk. For colostrum, breast milk–derived macrophages are the most populated cell type (25.88%–37.92%). Lymphocytes accounted for 14.16% to 23.57%, which is slightly higher than what was reported.⁴ We noticed that NK cells have the highest percentage among lymphocytes in our study population, whereas T cells are the major players reported in human milk (>80%).⁴

Neonates are prone to infectious and allergic diseases because of their immature immune system. Accumulating evidence has suggested that exclusive breastfeeding until 6 months of age may reduce the incidence of infectious diseases and allergy in early childhood.^{7–9} Characterizing the cellular components of breast milk will facilitate the understanding of such immunological role of this biological body fluid, which is involved in both mucosal defense of breastfed infant and viral transmission.¹⁰

Our data show that the total cell counts in human colostrum ($519\text{--}667 \times 10^6/\text{L}$) are less than those reported in the literature ($4 \times 10^9/\text{L}$).⁴ Literature concerning the immunocompetent cells in human milk has reported variable results based on country of origin. Eglinton et al¹¹ reported that the total cell count of 20 samples of colostrum (4.6 $\pm$ 1.4 days after delivery) was $0.67 \pm 0.52 \times 10^6/\text{mL}$, which yielded 7.5% $\pm$ 6.5% lymphocytes ($0.05 \times 10^6/\text{mL}$), 61.5% $\pm$ 12.6% macrophages, and 28.5% $\pm$ 15.1% neutrophils. Järvinen and Suomalainen⁶ found that the cellular composition of human milk correlated with cow's milk allergy in breastfed infants. The phenotype and features of immunocompetent cells in human milk in Chinese population have not been reported.

The significance of a high percentage of breast milk–derived macrophages is still controversial. Breast milk–derived macrophages (CD14⁺) are considered the storage mechanism for human IgA. They can regulate infant immune system through the expression of activation markers (eg, CD11c) that interact with infant T and B cells. These cells also secrete immunoregulatory factors such as lysozyme, lactoferrin, peroxidase, complex oligosaccharides, fatty acids, and mucins when they are in contact with bacteria in the gut.^{12,13} Järvinen and Suomalainen⁶ found that human milk macrophages express high levels of human leukocyte antigen–DR and

produce a significant amount of tumor necrosis factor- α . However, such expression and production are compromised in mothers of infants with cow's milk allergy, suggesting that the central role of human milk macrophages might be to induce oral tolerance in the breast-feeding infant.⁶

NK cells and cytolytic T lymphocytes recognize tumor cells or virally infected cells through interaction with unique antigens expressed on the cell surface. They then kill infected cells via mechanisms involving perforin and granzymes. Many studies⁷ suggest an association between the deficiency of NK cell activity and the increased susceptibility to herpes simplex virus and human immunodeficiency virus infection in preterm infants and newborns. The relatively higher percentage of NK cells and cytolytic T lymphocytes in human breast milk may compensate for the deficiency. Interleukin-22–producing NK cells, a novel human natural killer cell subset, which were found in lamina propria, may play a pivotal role in maintaining mucosal immunity and tissue repair.^{14,15} Clinical reports have shown that some children with severe combined immunodeficiency developed graft versus host disease when they were started on breast milk,¹ implying that some penetration of cells through gut epithelia takes place during normal breastfeeding. We speculate that the high percentage of NK cells in human milk may play a critical role in maintaining mucosal homeostasis and inducing immune tolerance. Newborns have a skewed CD4⁺ subpopulation, with Th2 cells being dominant. This may make them prone to allergic diseases. The higher numbers of T and NK cells in maternal colostrum may provide a compensatory mechanism to overcome such skew. This results in a relative lower percentage of CD19⁺ cells in colostrum.

Our data showed that CD4⁺ T cells in colostrum express CD40L, CD25 (IL-2R α), and CD45RO without any stimulation, which is consistent with reports in the literature.^{11,16,17} It can be assumed that such CD4⁺ T lymphocytes in colostrum are activated memory T cells. This is beneficial to infants, whose immune system is immature.

CD45RA⁺CD45RO⁺ subset of CD4 T cells is considered a transition form of cells from naive to effector phenotype during CD4 priming with increased expression of activation markers. The generation of this subset can occur in 2 ways: the transition of recently activated of CD45RA⁺ T naive cells to CD45RO⁺CD45RA⁺ phenotype, and re-expression of CD45RA⁺ isoform in the absence of antigen stimulation.¹⁸ The number of cells belonging to this subset is far greater in secondary

lymphoid organs than in the circulation. In the periphery, these cells are found at a low frequency (<1%).¹⁹ Our study showed that the CD45RA⁺CD45RO⁺ subset of CD4 T cells exists in both colostrum and mature human milk. We speculate that this subset may be derived from CD45RO⁺ cells given the lack of Ag stimulation in colostrum and mature human milk. However, the functional significance of this subset in milk needs to be further explored.

We analyzed the association of atopy and cesarean delivery with the distribution of lymphocytes in human colostrum. The numbers of NK cells and memory T cells in breast milk from atopic mothers were significantly lower than in mothers without atopy. Some studies revealed that the cytokines and biochemical composition of colostrum differ significantly between mothers with and without atopic diseases,^{20,21} although Järvinen and Suomalainen⁶ showed no such difference. NK cells play a critical role in anti-infection, killing tumors and preventing autoimmune diseases. Recent reports, however, suggest an association between NK cells and allergic diseases.²²⁻²⁴ Three regulatory pathways may be used by activated NK cells to inhibit the development of Th2 response to allergens. First, cytokines such as interferon- γ that are secreted by activated NK cells may create a microenvironment that promotes the development of Th1 cells and/or prevents the naive T cells from differentiating into the Th2 subtype. Second, NK cells inhibit allergic responses via direct interaction with T cells or other inflammatory cells. Activated human NK cells express MHC class II, B7, and OX40L, which interact with T-cell markers such as TCR, CD28, and OX40, respectively, resulting in T-cell proliferation, cytokine production, and immune memory generation. Third, activated NK cells may modulate allergen-specific T-cell responses by interacting with dendritic cells (DCs), a key player in initiating and shaping adaptive immune responses upon allergen exposure. NK cells may stimulate CD8 α ⁺ DCs, the DC subset with greater effect in inducing Th1 responses.²⁵ Naive CD45RA⁺ T cells, upon Ag priming, develop into CD45RO⁺ memory-type T cells. These memory-type T cells protect the host from infectious diseases through the production of cytokines. CD45RO⁺ memory-type T cells can also induce Th1 responses.²⁶ Animal studies have shown that lymphocytes in colostrum can be absorbed directly by neonates through small intestine mucous membrane. Breast milk-derived immunocompetent cells are found in mesenteric nodes and spleens of rodent pups, lambs, and neonatal baboons.²⁷ Although there are only a few studies in humans, the relative neutral pH of

the stomach in early infancy, together with the buffering capacity of milk, may allow survival of milk lymphocytes as well as cytokines during gastrointestinal transit. A human organ culture system demonstrated that human milk cells are capable of traversing the gut epithelium in the presence of milk.²⁸ Accumulated evidence suggests that breastfeeding is accompanied by a trafficking of maternal cells to the infant's lymphoid tissues. Since T cells and some B cells have relatively long intermitotic times, they may be present in the recipient's system for many months to years.²⁹ This may explain the fact that infants of atopic mothers have increased risk of developing atopic diseases.

The number of activated CD4⁺ T lymphocytes is significantly reduced in colostrum from mothers who undergo cesarean delivery compared with mothers with spontaneous vaginal delivery. It is widely accepted that cesarean delivery not only can increase short-term adverse outcomes in infants such as respiratory distress but also is associated with risks of developing food allergy, obesity, asthma, and type 1 diabetes in childhood or adulthood.³⁰ Recently, Sánchez-Valverde et al³¹ confirmed that cesarean delivery is associated with IgE-mediated cow's milk allergy. There have been no reports in the literature regarding the association of cesarean delivery with the composition of immunocompetent cells in human milk. The components of human breast milk vary greatly throughout the lactation period. During gestation, primeval milk accumulates in mammary glands and then becomes colostrum until labor occurs. The mode of labor presentation can influence lactation time.

Our studies had limitations because the sample size was relatively small and the percentage of mothers with cesarean delivery was relatively higher than that previously reported (>60%), which may be related to the overall lower number and percentage of immunocompetent cells in the milk of Han Chinese women.

As demonstrated in this study, the cellular composition of human milk in Han Chinese differs from that reported in other countries. The total cell number is slightly lower and the percentage of lymphocytes is higher than those reported. Furthermore, the highest percentage of lymphocytes in human milk consists of NK cells instead of T cells as shown in the literature. In agreement with data reported, the main population in CD4⁺ T lymphocytes consists of activated memory T cells. CD45RA⁺CD45RO⁺ subset of CD4 T cells exists in both colostrum and mature human milk. Atopy and cesarean delivery may be associated with the phenotypes of lymphocytes in colostrum, which needs to be further elucidated by multicenter studies

with large sample sizes. The functional aspect of these large amount cells in human milk deserves further exploration.

References

1. Michie CA. The long term effects of breastfeeding: a role for the cells in breast milk? *J Trop Pediatr*. 1998;44:2-3.
2. Kneebone GM, Kneebone R, Gibson RA. Fatty acid composition of breast milk from three racial groups from Penang, Malaysia. *Am J Clin Nutr*. 1985;41:765-769.
3. De Amici D, Gasparoni A, Guala A, Klersy C. Does ethnicity predict lactation? A study of four ethnic communities. *Eur J Epidemiol*. 2001;17:357-362.
4. Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. *Pediatr Infect Dis J*. 1993;12:664-671.
5. Kelly D, Coutts AG. Early nutrition and the development of immune function in the neonate. *Proc Nutr Soc*. 2000;59:177-185.
6. Järvinen KM, Suomalainen H. Leukocytes in human milk and lymphocyte subsets in cow's milk-allergic infants. *Pediatr Allergy Immunol*. 2002;13:243-254.
7. Lawrence RM, Pane CA. Human breast milk: current concepts of immunology and infectious diseases. *Curr Probl Pediatr Adolesc Health Care*. 2007;37:7-36.
8. Savilahti E. Interaction of early infant feeding, heredity and other environmental factors as determinants in the development of allergy and sensitization. *Nestle Nutr Workshop Ser Pediatr Program*. 2008;62:157-168; discussion 168-172.
9. Oddy WH. The long-term effects of breastfeeding on asthma and atopic disease. *Adv Exp Med Biol*. 2009;639:237-251.
10. Lawrence RM, Lawrence RA. Breast milk and infection. *Clin Perinatol*. 2004;31:501-528.
11. Eglinton BA, Robertson DM, Cummins AG. Phenotype of T cells, their soluble receptor levels, and cytokine profile of human breast milk. *Immunol Cell Biol*. 1994;72:306-313.
12. Rivas RA, el-Mohandes AA, Katona IM. Mononuclear phagocytic cells in human milk: HLA-DR and Fc gamma R ligand expression. *Biol Neonate*. 1994;66:195-204.
13. Brandtzaeg P. Mucosal immunity: integration between mother and the breast-fed infant. *Vaccine*. 2003;21:3382-3388.
14. Malmberg KJ, Ljunggren HG. Spotlight on IL-22-producing NK cell receptor-expressing mucosal lymphocytes. *Nat Immunol*. 2009;10:11-12.
15. Cella M, Fuchs A, Vermi W, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature*. 2009;457:722-725.
16. Gibson CE, Eglinton BA, Penttilä IA, Cummins AG. Phenotype and activation of milk-derived and peripheral blood lymphocytes from normal and coeliac subjects. *Immunol Cell Biol*. 1991;69:387-393.
17. Wirt DP, Adkins LT, Palkowetz KH, Schmalstieg FC, Goldman AS. Activated and memory T lymphocytes in human milk. *Cytometry*. 1992;13:282-290.
18. Trushin SA, Bren GD, Badley AD. CD4 T cells treated with gp120 acquire a CD45RO+/CD45RA+ phenotype. *Open Virol J*. 2009;3:21-25.
19. Hamann D, Baars PA, Hooibrink B, van Lier RW. Heterogeneity of the human CD4+ T-cell population: two distinct CD4+ T-cell subsets characterized by coexpression of CD45RA and CD45RO isoforms. *Blood*. 1996;88:3513-3521.
20. Böttcher MF, Jenmalm MC, Garofalo RP, Björkstén B. Cytokines in breast milk from allergic and nonallergic mothers. *Pediatr Res*. 2000;47:157-162.
21. Böttcher MF, Jenmalm MC, Björkstén B, Garofalo RP. Chemoattractant factors in breast milk from allergic and nonallergic mothers. *Pediatr Res*. 2000;47:592-597.
22. Simon D, Kozlowski E, Simon H. Natural killer T cells expressing IFN-gamma and IL-4 in lesional skin of atopic eczema. *Allergy*. 2009;64:1681-1684.
23. Carbone T, Nasorri F, Pennino D, et al. CD56highCD16-CD62L-NK cells accumulate in allergic contact dermatitis and contribute to the expression of allergic responses. *J Immunol*. 2010;184:1102-1110.
24. Matangkasombut P, Pichavant M, Dekruyff RH, Umetsu DT. Natural killer T cells and the regulation of asthma. *Mucosal Immunol*. 2009;2:383-392.
25. Han X, Fan Y, Wang S, Jiao L, Qiu H, Yang X. NK cells contribute to intracellular bacterial infection-mediated inhibition of allergic responses. *J Immunol*. 2008;180:4621-4628.
26. Matsuyama T, Urano K, Ohkido M, et al. The quantitative and qualitative defect of CD4+ CD45RO+ memory-type T cells are involved in the abnormality of TH1 immunity in atopic dermatitis patients. *Clin Exp Allergy*. 1999;29:687-694.
27. Jain L, Vidyasagar D, Xanthou M, Ghai V, Shimada S, Blend M. In vivo distribution of human milk leukocytes after ingestion by newborn baboons. *Arch Dis Child*. 1989;64:930-933.
28. Michie CA, Harvey D. Maternal milk lymphocytes engraft the fetal gut. *Pediatr Res*. 1995;37:129A.
29. McLean AR, Michie CA. In vivo kinetics of lymphocyte subsets. *Proc Natl Acad Sci U S A*. 1995;92:3707-3711.
30. Steer PJ, Modi N. Elective caesarean sections-risks to the infant. *Lancet*. 2009;374:675-676.
31. Sánchez-Valverde F, Gil F, Martínez D, et al. The impact of caesarean delivery and type of feeding on cow's milk allergy in infants and subsequent development of allergic march in childhood. *Allergy*. 2009;64:884-889.