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POPULATION STUDY ARTICLE

Dynamic change, influencing factors, and clinical impact of cellular components in human breast milk

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BACKGROUND: Numerous cellular components have been well demonstrated in human breast milk. However, little is known about their dynamic change, influencing factors, and potential clinical impacts on infants.
METHODS: Sixty and forty-five healthy mother–infant pairs were enrolled in the colostrum group and mature milk group, respectively. Participants' demographic and clinical information were collected by questionnaires, and the infants were followed up until 6 months after birth through telephone interview. Colostrum and mature milk were collected, and the percentage of various cell components were determined by flow cytometric analysis.
RESULTS: The results showed that, the total cell numbers, and the percentages of some stem cells, including CD34+, CD117+, CD133+, CD90+, CD105+, and CD146+ cells, were different in colostrum and mature milk. Besides, participants' characteristics had influence on the cellular components. Finally, high-CD34+ cells in colostrum, as well as the high-CD133+ cells and low-CD105+ cells in mature milk were associated with a significantly increased risk of infantile eczema within their first 3 months after birth.
CONCLUSIONS: Our data showed a dynamic change of cellular components, identified some of their influencing factors and their potential clinical impacts on infantile eczema, which helps to better understand the cellular components in human breast milk.

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IMPACT:

- Some stem cell markers were dynamically changed in human colostrum and mature milk.
- Different cellular components were shown to be influenced by different participants' characteristics.
- High percentage of CD34+ cells in colostrum, as well as high percentage of CD133+ cells and low percentage of CD105+ cells in mature milk, were associated with a significantly increased risk of infantile eczema within their first 3 months after birth.
- To our knowledge, this is the first study on the clinical impacts of stem cells on infantile diseases, which helps to give a better understanding of human breast milk.

INTRODUCTION

Human breast milk, which contains a number of diverse components, is considered to be the optimal food for the human infants.[1,2] In addition to proteins, lipids, and carbohydrates, which provide essential nutrition to the infants, human breast milk contains a diverse biologically active components, microbiome, and a heterogeneous population of cells.[3] Human breast feeding has been well demonstrated to be not only associated with short-term but also long-term substantial benefits for the children, including protecting against various infections in their early life, promoting gastrointestinal and neurological development and maturation, reducing the risk of numerous diseases during their adolescence and adulthood, such as obesity, hypertension, type 2 diabetes, cardiovascular disease, atopic disease, and so on.[2,4–7]

In the past decades, numerous cell populations have been demonstrated in human breast milk, including leukocytes, lactocytes, myoepithelial cells, progenitor and stem cells, and so on. The presence of stem cells in human breast milk was first demonstrated in 2007.[8] The discovery of stem cells with multilineage differentiation potential in human breast milk makes them potential materials in the further regenerative medicine. Besides, breast milk stem cells can also be used in studies that aim to understand the biology of the lactating breast as well as the pathology of abnormality during lactation.[9]

In the past decades, accumulating evidences have demonstrated that human breast milk ingredients were dynamic, which could be changed with the stage of lactation. Besides, components in breast milk were shown to be affected by the degree of

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breast fullness, the health of mothers and infants, infant feeding, and so on.[10] However, to date, studies on factors influencing the cellular components in breast milk are still limited.

It was reported that leukocytes in human breast milk could transfer from mothers to their infant's bodies, which was known as a process called microchimerism.[11,12] Following studies showed that some breast milk progenitor cells were able to penetrate the infant's gastrointestinal tract, entering their circulatory system, then populating distant organs.[9,13] Therefore, it is reasonable for some researchers to hypothesize that breast milk stem cells could interact with the infants, promote their tissue homeostasis, development, and overall regeneration.[14] However, studies on the physiological roles and health implications of various cells in human breast milk are still limited, and further studies are still needed to unveil it.

Herein, we aimed to study the dynamic change of some cellular components in human colostrum and mature milk, as well as the influence of participant characteristics on these cellular components. Furthermore, effects of these cellular components in human milk on three common infantile disease (including eczema, upper respiratory infection (URI), and diarrhea) within the first 6 months after birth were evaluated. Elucidating the dynamic change, influencing factors, and clinical effects of cellular components in human breast milk will help to understand its critical role in human breast feeding.

MATERIAL AND METHODS

Subjects and data collection

From September 2018 to March 2020, we recruited 60 and 45 healthy mother–infant pairs in colostrum and mature milk cohorts in Shanghai Punan Hospital, Shanghai, China, respectively. All subjects enrolled were Han Chinese. Inclusion criteria were as follows: healthy mothers who had no history of chronic diseases, no acute infections and pregnancy complications during the gestation and lactation periods; healthy infants who passed clinical assessment. The cesarean sections were because of mothers' attitudes toward mode of delivery and the fetal malposition. This study was approved by the Ethical Committee of Shanghai Children's Medical Center and Shanghai Punan Hospital, and written informed consent was obtained from all the participants. Questionnaire were used to collect the mothers' and their infants' demographic and clinical information. Mothers' information included mothers' age, height, mode of delivery, parity, history of allergy, blood glucose, serum total protein, pre-pregnancy body mass index (BMI), prenatal BMI, and post-partum BMI. Infants' information included sex, gestational age, and birth weight.

Infants were followed up at 3 months and 6 months after birth through telephone interview. The occurrence of three common infantile diseases, including eczema, URI, and diarrhea, was recorded to evaluate the potential clinical impacts of cell components on infants.

Sample collection

Colostrum was collected within 5 days after delivery, and mature milk was collected at 42 days postpartum. All samples were collected by pumping all milk from one side of breast at a time, then transferred on ice to laboratory for further studies.

Detection of serum total protein

The serum total protein measured all the protein levels in the mothers' peripheral blood, which was quantitatively analyzed by the biuret method on an ARCHITECT c16000 clinical chemistry analyzer.

Flow cytometric analysis

The cellular components of human breast milk were isolated as previously described.[15] Briefly, 1 mL breast milk samples were centrifuged at 805 × *g* for 20 min. The upper fat layer and the whey were removed, and the cell pellet was washed twice with phosphate-buffered saline (PBS). Then the cell pellet was resuspended using 1 mL PBS, and trypan blue exclusion tests were used to determine the number of live and dead cells. The median percentage of dead cells in the colostrum is 54.29% (40.11–69.94%), and the median percentage of dead cells in the colostrum is 48.12% (28.10–71.86%).

After that, flow cytometry was used to detect the markers on the human breast milk cells. One hundred microliters of cell suspension were stained for 20 min in the dark with 5 μL of the following labeled antibodies: Alexa Fluor® 647 Anti-Cytokeratin 5, Alexa Fluor® 488 Anti-Cytokeratin 18 (Abcam), CD34-PE-cy7, CD45-APC-H7, CD117-PE, CD133-V450, CCR6-PE, CD105-APC, CD44-PE, CD73-APC, CD90-BV450, CD146-BV421, and CD271-PE-cy7 (BD Pharmingen). Samples were analyzed using a BD FACSCanto II system (BD Pharmingen), and data were analyzed using the FlowJo software (Tree Star, Inc.)

Statistical analysis

Differences in participants' characteristics in the colostrum group and mature milk group were determined using Mann–Whitney *U*-test, *t* test, or χ^2 test. Differences of the percentages of various cell components at different stages of lactation were evaluated by Mann–Whitney *U*-test. Correlation analysis on cell markers in human breast milk and the participants' characteristics was conducted by Spearman's or point-biserial correlation analysis. Multiple linear regression analysis was used to identify the influencing factors of participants' characteristics on the cellular components of human breast milk. Binary logistic regression analysis was used to identify the effects of cell components on the infantile diseases. Statistical analyses were performed using IBM SPSS Statistics 26. All hypotheses were two-sided, and *P* values < 0.05 were considered statistically significant.

RESULTS

Characteristics of the mothers and their infants

A total of 60 colostrum and 45 mature milk samples were collected in this study. The characteristics of the participants in each group are listed in Table 1, and there was no significant difference between the two groups. Three infants in the colostrum group and 1 infant in the mature milk group had a gestational age ≤37 weeks, and there was no significant between the two groups ($P = 0.424$).

Identification of stem cells in human colostrum and mature milk

Total cell numbers were determined in both of the colostrum and mature milk groups. As shown in Table 2, the total cell number was significantly higher in the colostrum group than that in the mature milk group ($P < 0.001$), which was consistent with the previous reports.

Hemopoietic stem cells (CD34+, CD117+, and CD133+), hematopoietic cells (CD45+), mesenchymal stem cells (MSCs) (CD90+, CD73+, CD44+, CD105+, CD271+, and CD146+), myoepithelial cells (CK5), lactocytes (CK18) and chemokine receptor (CCR6) were identified in human breast milk by flow cytometry. As shown in Table 2, compared with the colostrum group, the percentages of CD34+, CD117+, CD133+, CD90+, CD105+ and CD146+ cells were significantly higher in the mature milk group.

These data suggested that, not only the total cells but also many stem cells varied in the different stages of lactation.

The influence of participant characteristics on the cellular components in human breast milk

To identify the influence of maternal and infantile characteristics on the cellular components in human breast milk, we first evaluated the correlations between participant characteristics and cellular components.

In the colostrum group, the results showed that the total cell numbers and the proportion of CD45+ and CK5+ cells was negatively related with the maternal age ($rs = -0.269$, -0.406, and -0.401, $P = 0.043$, 0.005, and 0.005, respectively), the proportion of CD90+ cells was positively related with the pre-pregnancy BMI and prenatal BMI ($rs = 0.367$ and 0.466, $P = 0.018$ and 0.002, respectively), the proportion of CD44+ cells and CCR6+ cells was positively related with the maternal blood glucose ($rs = 0.475$ and 0.340, $P = 0.008$ and 0.029, respectively), the proportion of CD271+ cells was positively related with the infantile birth weight ($rs = 0.403$, $P = 0.025$) (Supplementary Table 1).

Table 1. The characteristics of the infants and their mothers included in the study.

Factors	Colostrum ($n = 60$)	Mature milk ($n = 45$)	*P* value
Maternal characteristics			
Age (years)	29.75 ± 4.65	30.06 ± 4.8	0.742
Mode of delivery, *n* (%)			
Vaginal	23 (38.98%)	19 (43.18%)	0.847
Cesarean	36 (61.02%)	25 (56.82%)	
Parity, *n* (%)			
1	40 (67.80%)	30 (68.18%)	0.967
2	19 (32.20%)	14 (31.82%)	
History of allergy, *n* (%)			
No	56 (93.33%)	42 (93.33%)	0.658
Yes	4 (6.67%)	3 (6.67%)	
Blood glucose (mmol/L)	4.29 ± 0.48	4.35 ± 0.53	0.63
Serum total protein (g/L)	58.22 ± 4.18	58.48 ± 4.21	0.763
Pre-pregnancy BMI (kg/m^2)	20.56 ± 2.79	20.58 ± 2.84	0.98
Prenatal BMI (kg/m^2)	26.20 ± 3.52	26.17 ± 3.51	0.975
Post-partum BMI (kg/m^2)	23.00 ± 4.52	23.04 ± 4.98	0.895
Infantile characteristics			
Sex			
Male	33	25	0.955
Female	27	20	
Gestational age (weeks)	39.03 ± 1.25	39.22 ± 1.19	0.467
Birth weight (g)	3257.33 ± 505.19	3330.56 ± 533.71	0.475
Feeding patterns before 3 months of age			
Exclusively breastfeeding	38 (70.37%)	34 (79.07%)	
Mixed feeding	3 (5.56%)	0 (0.00%)	0.2973
Formula feeding	13 (24.07%)	9 (20.93%)	
Feeding patterns before 6 months of age			
Exclusively breastfeeding	27 (67.5%)	27 (67.5%)	–
Mixed feeding	8 (20%)	8 (20%)	
Formula feeding	5 (12.5%)	5 (12.5%)	

BMI Body Mass Index.

In the mature milk group, the results showed that the proportion of CD34+ cells was positively related with the serum total protein ($rs = 0.381$, $P = 0.024$), the proportion of CD117+ cells was positively related with the serum total protein ($rs = 0.429$, $P = 0.01$), but negatively related with the prenatal BMI ($rs = -0.351$, $P = 0.036$), the proportion of CD133+ and CD45+ cells was positively related with the serum total protein ($rs = 0.486$ and 0.447, $P = 0.003$ and 0.007, respectively), the proportion of CD44+ and CD271+ cells was positively related with the infantile sex ($rs = 0.353$ and 0.371, $P = 0.028$ and 0.02, respectively), the proportion of CD146+ and CK18+ cells was positively related with the maternal serum total protein ($rs = 0.389$ and 0.388, $P = 0.023$ and 0.021, respectively), and the proportion of CCR6+ cells was negatively related with the pre-pregnancy BMI, prenatal BMI, post-partum BMI, and infantile birth weight ($rs = -0.580$, -0.447, -0.462 and -0.401, $P < 0.001$, $P = 0.006$, 0.005, and 0.01, respectively) (Supplementary Table 2).

The influence of participant characteristics on the cellular components of human breast milk

Subsequently, statistically significant ($P < 0.2$) factors in the correlation analysis mentioned above were selected, and a further multiple linear regression with backward selection (threshold, $P < 0.05$) was used to evaluate the influence of participant characteristics on the cellular components of human breast milk. The results of multiple linear regression analysis are shown in Table 3. It was shown that an advanced maternal age was associated with a lower total cell number and a lower proportion of CD45+ and CK5+ cells in colostrum. Besides, in colostrum, a higher prenatal BMI was associated with a higher proportion of CD90+ cells, a higher maternal blood glucose was associated with a higher proportion of CD44+ cells, a higher infantile birth weight was associated with a higher proportion of CD271+ cells, and a more parity was associated with a lower proportion of CD146+ cells.

However, factors influencing cellular components in human mature milk were shown to be different from that in the colostrum. In mature milk, a higher serum total protein was associated with a higher proportion of CD117+, CD133+, CD146+, and CK18+ cells, a higher prenatal BMI was associated with a lower proportion of CD117+ and CCR6+ cells, and female infant was associated with a lower proportion of CD44+ and CD271+ cells (Table 3).

The effect of cellular components in human milk on infantile diseases within the first 6 months after birth

To evaluate the effect of cellular components in human milk on infantile disease, total cell numbers and various cellular components

Table 2. Percentages of different cell components in human milk at different stages of lactation.

Classification		Colostrum	Mature milk	*P* value
		Median (IQR, *n*)		
	Total cell numbers****	1,901,429 (580,917, 6,002,500, 58)	90,500 (40,200, 266,420, 41)	<0.001
Hematopoietic stem/hematopoietic cell markers	CD34****	17.74 (13.09, 25.33, 47)	30.47 (23.61, 36.15, 40)	<0.001
	CD117*	7.29 (5.51, 19.76, 31)	15.18 (9.04, 19.84, 40)	0.02
	CD133**	10.51 (6.51, 15.16, 47)	18.71 (9.00, 28.22, 40)	0.005
	CD45	10.61 (6.10,14.14, 47)	9.35 (4.10, 16.93, 40)	0.54
Mesenchymal stem cell markers	CD90****	3.20 (1.33, 6.35, 46)	9.45 (6.67, 11.53, 39)	<0.001
	CD73	1.50 (0.29, 2.18, 31)	1.67 (1.00, 3.71, 39)	0.147
	CD44	6.28 (3.97, 12, 31)	5.83 (3.62, 9.67, 39)	0.732
	CD105**	9.50 (5.64, 17.59, 31)	23.85 (9.47, 33.50, 40)	0.009
	CD271	21.46 (16.42, 28.43, 31)	25.49 (20.84, 29。65, 39)	0.159
	CD146****	1.66 (0.73, 4.4, 31)	9.52 (6.01, 15.88, 39)	<0.001
Myoepithelial cell marker	CK5	10.57 (5.74, 24.2, 47)	14.68 (8.62, 23.26, 40)	0.204
Lactocyte marker	CK18	7.87 (3.98, 18.55, 47)	11.13 (6.5, 22.13, 40)	0.09
Chemokine receptor	CCR6	3.87 (1.09, 14.94, 47)	5.43 (2.7, 23.87, 40)	0.123

IQR interquartile range.

in colostrum and mature milk were divided into high and low groups based on their medians. Due to the vital role of family history of allergy and food antigens for the development of infantile diseases, especially for the development of infantile atopic eczema, maternal history of allergy and the infantile feeding patterns were also included in the further correlation analysis.

Thereafter, we evaluated the correlations between various cellular components, maternal history of allergy, infantile feeding patterns, and infantile diseases (including eczema, URI, and diarrhea) within the first 3 and 6 months after birth. The result showed that, in the colostrum group, the feeding patterns significantly correlated with infantile eczema within their first 3 months after birth (Kendall's tau-b = −0.317, $P = 0.018$), indicating that exclusively breastfeeding might have a protective role in the infantile atopic eczema that develops within their first 3 months after birth (Supplementary Table 3). Besides, maternal history of allergy was positively correlated with infantile diarrhea within their first 3 months after birth (Kendall's tau-b = −0.312, $P = 0.023$). In the mature milk group, as shown in Supplementary Table 4, maternal history of allergy positively correlated with infantile URI within their first 3 months after birth (Kendall's tau-b = 0.317, $P = 0.048$), and the high-CD271+ group positively correlated with infantile diarrhea within their first 6 months after birth (Kendall's tau-b = 0.391, $P = 0.022$).

Then, statistically significant ($P < 0.2$) factors in the correlation analysis mentioned above were selected, and a further binary logistic regression with backward selection (threshold, $P < 0.05$) was used to evaluate the effects of cellular components in human milk on infantile diseases within the first 3 and 6 months after birth. As shown in Table 4, it was found that the high-CD34+ group in colostrum, as well as the high-CD133+ group and the low-CD105+ group in mature milk, was associated with a significantly increased risk of infantile eczema within their first 3 months after birth.

DISCUSSION

Human breast milk contains numerous immunological, biochemical, and cellular components that could significantly affect newborns' development, immunity, and susceptibility to various diseases.[16,17] For the cellular components, although their presence in human breast milk has been well demonstrated, less is known about the proportion of different cell types and their influencing factors, as well as their significance to the infants.[3,9] In this study, we sought to determine the dynamic change of cellular components, as well as their influencing factors and their potential clinical impacts on infants. Three major results were observed: (i) Total cell numbers and many stem cells (CD34+, CD117+, CD133+, CD90+, CD105+, and CD146+ cells) varied in the different stages of lactation. (ii) Participants' characteristics had influence on the cellular components in human breast milk, and factors influencing cellular components in mature milk were different from that in the colostrum. (iii) High percentage of CD34+ cells in colostrum, as well as the high percentage of CD133+ cells and low percentage of CD105+ cells in mature milk, was shown to be associated with a significantly increased risk of infantile eczema within their first 3 months after birth.

Breast milk was previously thought to contain epithelial cells and immune cells until Cregan's groundbreaking discovery of stem cells in human breast milk in 2007.[8] Typically, maternal-derived cells in human breast milk could be divided into two groups: blood-derived and breast-derived cells. Blood-derived cells mainly include immunological cells and hematopoietic cells, and breast-derived cells are mainly comprised of lactocytes and myoepithelial cells, both of which were reported to contain progenitor or stem cells.[10] As other components in the breast milk, cellular components could change dynamically both within and between women.[17] It was shown that the total cell number in the colostrum was significantly higher in the colostrum than that in mature milk in this study, as well as in the previous reports.[15,18]

Compared with other cellular components, leukocytes in breast milk have received more attention because of their potential benefits of protecting infants from infections. Accumulating evidences demonstrated that breast milk leukocytes could increase when breastfeeding mothers or infants had infections, indicating that there is a tightly controlled process aiming to confer extra immunological support to the infants.[3,19,20] In addition to leukocytes, hematopoietic stem/progenitor cells, including CD34+, CD117+, CD133+, and so on, were also reported to be present in human breast milk.[21–23] However, there is still a debate on the percentage of these maternal blood-derived cells in colostrum and mature milk. Goudarzi et al. reported that the percentages of CD34+, CD117+, and CD133+ were all significantly higher in colostrum than that in the mature milk.[24] However, Li et al. reported that the percentage of CD34+ cells were significantly higher in

Table 3. Identification of the influence of participant characteristics on the cell composition of human breast milk by multiple linear regression analysis.

Variables	Factors	β	P	95% CI for β	
				Lower bound	Upper bound
Colostrum					
Log(Cell number)	Maternal age	−0.315	0.017	−0.07	−0.007
Log(CD45)	Maternal age	−0.458	0.001	−0.048	−0.013
Log(CD90)	Prenatal BMI	0.427	0.006	0.02	0.111
Log(CD44)	Blood glucose	0.384	0.036	0.026	0.733
Log(CD271)	Birth weight	0.415	0.02	0	0
Log(CD146)	Parity	−0.413	0.023	−1.014	−0.08
Log(CK5)	Maternal age	−0.367	0.018	−0.065	−0.006
Mature milk					
Log(CD117)	Serum total protein	0.357	0.036	0.002	0.048
	Prenatal BMI	−0.384	0.025	−0.066	−0.005
Log(CD133)	Serum total protein	0.371	0.028	0.003	0.053
Log(CD44)	Sex	0.448	0.008	0.083	0.506
Log(CD271)	Sex	0.462	0.009	0.029	0.187
Log(CD146)	Serum total protein	0.339	0.005	0	0.072
Log(CK18)	Serum total protein	0.341	0.045	0.001	0.055
Log(CCR6)	Prenatal BMI	−0.64	<0.001	−0.204	−0.084

β standardized coefficients.

Table 4. Effect of CD34+, CD133, and CD105+ cells in human breast milk against eczema during their first 3 months of life.

	OR (95% CI)	P value
CD34 in colostrum		
Low group (<17.74%)	1.00	
High group (≥17.74%)	6.222 (1.212–31.937)	0.028
CD133 in mature milk		
Low group (<18.71%)	1.00	
High group (≥18.71%)	7.194 (1.065–48.584)	0.043
CD105 in mature milk		
Low group (<23.85%)	1.00	
High group (≥23.85%)	0.075 (0.008–0.675)	0.021

CI confidence interval.

transitional and mature milk.[15] Our results were consistent with Li et al., which showed that the percentages of CD34+, CD117+, and CD133+ were all significantly higher in mature milk than that in the colostrum, which might be due to the same race (Han Chinese) enrolled in Li et al. and this study.

To date, studies on breast-derived cells in breast milk are still limited. Sani et al. demonstrated that purified human breast milk-derived stem cells could be differentiated toward different lineages, and a remarkable number of cells expressed the MSC markers, such as CD90, CD44, CD271, and CD146.[25] Other cell makers of MSCs were also reported to be present in human breast milk, including CD73, CD105, CD49f, and so on.[3,26] However, reports on differences in the percentage of these MSCs in colostrum and mature milk are limited and contradictory.[15,24] Many factors might contribute to the different results in different studies, which included race, ethnicity, environment, diets, culture, and so on. In this study, we showed that the percentages of CD90+, CD105+, and CD146+ cells were significantly higher in mature milk than that in colostrum. Further studies are still needed to determine their characteristics and roles, as well as their mechanisms of transfer from maternal blood into breast milk.

It has been well demonstrated that maternal and infant characteristics could affect human milk ingredients.[27–29] However, little efforts were made to identify influencing factors on cellular components in breast milk.[3,9,15,30] In this study, it was shown that maternal ageing had a negative influence on some cellular components in colostrum, including total cell numbers, and the percentage of CD45+ and CK5+ cells. This result was consistent with the previous reports which showed that a higher maternal age might have negative effects on the beneficial ingredients in breast milk, as well as the performance of offspring.[31,32] Besides, it was interesting to find that maternal blood glucose had a positive influence on the percentage of CD44+ cells in this study. In keeping with this, Hadarits et al. reported increased proportion of hematopoietic stem and progenitor cell population in cord blood of neonates born to mothers with gestational diabetes mellitus.[33] However, further studies showed that gestational diabetes mellitus might affect the differentiation potency of hematopoietic stem cells from umbilical cord blood.[34] Moreover, we found that maternal serum total protein levels, which could partially reflect the nutritional status of the mother, had significant positive influences on some cellular components in mature milk. Therefore, our results further indicated the potential interactions between maternal nutritional status and the implications for the long-term health of the offspring.

It is widely accepted that human breast milk contains numerous components which provide benefits to the breastfeeding infants.[3,6,10,35,36] Exploring their physiological roles and health implications is of particular interest. Our results showed that the high percentage of CD34+ cells in colostrum, as well as the high percentage of CD133+ cells and low percentage of CD105+ cells in mature milk were associated with a significantly increased risk of infantile eczema within their first 3 month after birth. In consistent with our results, previous reports showed that CD34+ hemopoietic progenitor cells were potent effectors to promote

allergic inflammation, by which they might act as proinflammatory effector cells by themselves and directly contribute to the allergic inflammation.[37–39] In the past decades, many active components in human breast milk have been shown to be associated with the development of allergic disease, including cytokines, immunoglobulins, growth factors, human oligosaccharides, polyunsaturated fatty acids, polyamines, and so on.[7,40] Among them, some components were shown to have a protective role against allergic disease, such as transforming growth factor-beta, soluble CD14, soluble IgA, interferon-gamma, interleukin (IL)-10, and IL-12 and polyunsaturated fatty acids (n-3 PUFAs), while some were shown to correlate with a higher risk of allergy development, such as IL-4, IL-5, IL-13, and n-6 PUFA.[41] In fact, eczema is a kind of inflammatory disease, and MSCs have been used as a therapeutic tool for the treatment of atopic dermatitis in some innovative clinical trials, because MSCs have been proved in mediating induction of tolerance.[42,43] Although we did not mention too much about the regenerative medicine in this manuscript, which is not the main goal of the manuscript, our results did provide useful clues for further attempt in using MSC therapy for atopic dermatitis.

Our studies still have some limitations. First, the number of the participants in this study was small, which might make the statistical analysis limited or skewed. Second, paired colostrum and mature milk would be better for this kind of cohort study, which could better reflect the changes of breast milk composition, their influencing factors, and consequence. Third, more efforts are still needed to explore the effects of cellular components in mature milk on infantile eczema.

In conclusion, we determined the dynamic change of cellular components, especially stem cells in human colostrum and mature milk, as well as its influencing factors and its potential clinical impacts on infants. To our knowledge, this is the first study on the clinical impacts of stem cells on the infantile diseases, which helps to give a better understanding of human breast milk.

DATA AVAILABILITY

The data that support the finding of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

T.-X.C., S.-M.W. contributed to the study conception and design. Material preparation and data collection and analysis were performed by J.W., Y.-Y.J., and Y.L. J.L. and J.X. contributed to sample collection, as well as the participants' demographic and clinical information collection. The first draft of the manuscript was written by J.W., and all authors commented on previous versions of the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Committee of the Shanghai Children's Medical Center and Shanghai Punan Hospital. Informed consent was obtained from legal guardians, and written informed consent was obtained from all parents or guardians of the participants.

ADDITIONAL INFORMATION

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