

Original Article

Exploring the effects of formula feeding on infant immune development in China: A prospective cohort study

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Background and Objectives: The worldwide exclusive breastfeeding rate is suboptimal and this study aims to evaluate effects on infant immune development of formula feeding. **Methods and Study Design:** A prospective study including 221 infants fed with breast milk or formula was conducted. At 3-month and 9-month, the concentrations of total immunoglobulin (Ig)G, IgM, IgA, IgG1, IgG2, interleukin (IL)-4, interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) were measured by using enzyme-linked immunosorbent assay (ELISA). Natural killer (NK) cell activity and lymphocyte transformation testing were conducted. Furthermore, the occurrence of infantile diarrhea, respiratory infections and allergic diseases were questioned. **Results:** The levels of total IgG ($Z=-3.21$, $p=0.001$), IgG1 ($Z=-2.12$, $p=0.034$), IFN- γ ($t=-2.09$, $p=0.039$) and NK cell activity ($t=-2.14$, $p=0.034$) were significant higher in formula-fed infants compared to breast-fed after 3 months. At 9-month, the levels of total IgG ($Z=-4.34$, $p<0.001$), IgA ($Z=-2.05$, $p=0.041$) and TNF- α ($t=-2.10$, $p=0.037$) of formula-fed infants were higher, but the lymphocyte stimulation index ($t=2.76$, $p=0.007$) was lower than breast-fed infants. While, no significant differences were found in the incidences of diarrhea and respiratory tract infection ($p>0.05$). **Conclusions:** This investigation suggested that formula- and breast-feeding have different contributions to infant immune development, but the formula feeding would not cause significantly increase of diarrhea and respiratory infections.

Key Words: formula feeding, breastfeeding, immune development, diarrhea rate, respiratory infection rate

INTRODUCTION

The infant undergoes a transition from the mother's utero environment to extra-uterine life and feeding is needed urgently. The World Health Organization and the United Nations Children's Fund recommended that infants should be exclusively breastfeeding within 6 months after birth, and continue to be breastfed up to 2 years of age and beyond together with the introduction of appropriate complementary foods.^{1,2} Despite the global recommendations, breastfeeding behaviors continue to be suboptimal in the 21st century.^{3,4} In China, a national representative survey in 2013 reported that the crude exclusive breastfeeding rate under 6 months was 20.7% (908/4381).⁵ Infant formula milk could be used as an alternative, in case that breastfeeding is not desired or possible for several reasons. When a substitute form of nutrition is required, there are many infant formula choices available in the market, with macronutrient and micronutrient compositions as similar as possible to human milk.^{6,7}

Compared with formula milk, human milk is uniquely tailored and suited to infants and breastfeeding confers immunological and nutritional benefits. Breast milk contains immune modulating components including but is not limited to IgA, human milk oligosaccharides (HMOs), lactoferrin, epidermal growth factor (EGF) and trans-

forming growth factor- β 2 (TGF- β 2), that are beneficial to newborns during maturation of their immune system.⁸ Breastfeeding may have a weak protective effect on common disruptions of the immune system, such as type 1 diabetes inflammatory bowel disease and atopic diseases, including allergies and asthma.⁹⁻¹¹ Moreover, numerous prospective cohort studies and population-based studies have represented a protective role of breastfeeding compared with formula feeding, in reducing the risk of diarrheal, otitis media and respiratory tract infections.¹²⁻¹⁵ Continuing with one example of the risk of diarrheal, Boone, Kelly M et al reported that formula feeding duration was associated with increased odds of diarrhea in the full sample unadjusted model, however, this result was not statistically significant in the adjusted mode.¹⁶ Although breast milk is undeniably the ideal feeding choice,

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advances in infant formula nutrition continue to shrink the gap in many infant outcomes.¹⁷ Since infant formula feeding options are incredibly vast and differ between cultures and periods of birth, the researchers in the field need to acknowledge that formula feeding is not a homogeneous exposure.¹⁷ As formula-fed infants should not be treated as a homogeneous group, the research results may remain diverse and needed to be further explored.

Whether growth and development especially immune system of infants fed with formula can catch up with breastfeeding babies and how early nutrition influences them, are worth to be solved. In this analysis, we focused on the immune development of infants fed with formula or breast milk, including specific and non-specific immune function. This evidence-based and information-rich design may contribute to a certain guiding basis for early feeding in our study area.

METHODS

Subjects

This community-based prospective cohort research was conducted in a rural area of Hunan Province from September 2010 to July 2011. The sample size was determined by cluster sampling, which included 221 healthy infants at age 10-days to 4-months. The study was approved by the Ethics Committee of the Hunan Provincial Center for Disease Control and Prevention. Of all the participants, written informed consent of our study was obtained from infants' patients and/or legal guardians.

Infants would be included those fed with formula or exclusive breast milk. The main exclusion criteria were mixed feeding of breast and formula milk. The baseline demographic characteristics (such as age, sex, birth weight and length, gestational age and feeding mode) and mother delivery mode were collected from all participants through the questionnaire. Two groups (exclusively breast-fed and formula-fed group) were obtained according to feeding method. Exclusively breast-fed which is defined as fed without any intake of other liquids or foods within 6 months. 126 formula-fed and 95 breast-fed in-

fants were obtained at baseline. The immune indicators and questionnaire would be assessed at 2 follow-ups times (after 3 months and 9 months). Assuming some infants didn't continue sole fed instead of mixed feeding and not contacted during the 3 months, the drop-out rate was 17.9% in breast-fed group and 7.9% in formula group after 3 months. According to feeding guidelines for infants, complementary foods should be introduced at 4 to 6 month of age, exclusive breast-fed infants would intake additional food. Then interrelated information was available through questionnaires after 9 months. Excluding the drop-out infants at the 2th follow-ups time, 100 formula-fed and 70 breast-fed infants meeting the criteria of the study were obtained for analysis (Figure 1).

Questionnaire

Information including mother delivery mode, infant basic situations, vaccination status and feeding method were obtained from face-to-face interviews. The milk powder brand and specific product name of infants fed with formula should be provided, as it may be helpful in our analysis of recipes. Guardians were asked whether their babies had suffered from a respiratory tract infection, diarrhea or allergic diseases in the previous month and had visited a doctor even hospitalization for the infectious disease. Information on respiratory tract infections was obtained by asking guardians whether their child had suffered from a serious cold, throat infection, bronchitis or pneumonia. Diarrhea was defined as three or more liquid stools in a 24-h period.¹⁸ These questions formed both binary (ever/never) and count (number of occurrences) variables for each infant for analysis.

Measurement of plasma immunoglobulins (Igs) and cytokines concentrations

Venous blood samples were drawn, heparinized and plasma separated stored at -80°C for further analysis. Plasma Igs and cytokines concentrations were measured using standard 96-well plate enzyme-linked immunosorbent assay (ELISA) kits according to the instruction

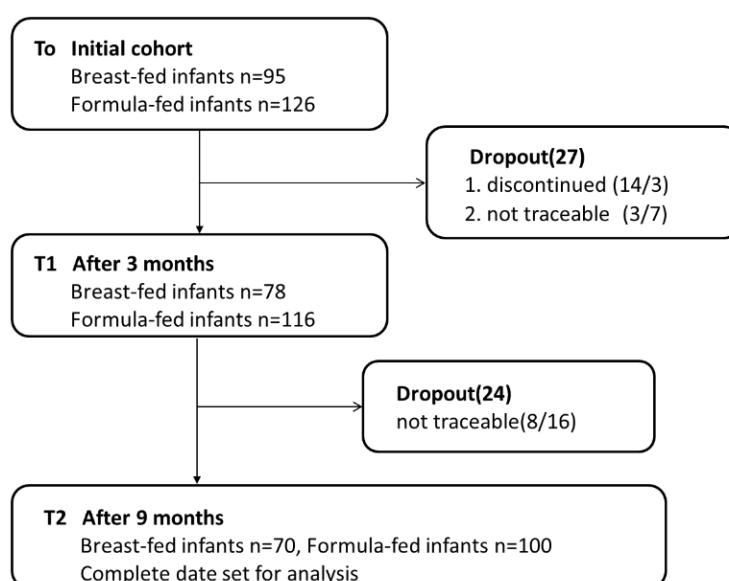


Figure 1. Study flow-chart.

manual [total IgG (detection range 0.15-15 ng/mL), IgM (detection range 1.56-100 ng/mL), IgG subtypes IgG1 (detection range 0.05-40 ng/mL) and IgG2 (detection range 1.56-100 ng/mL), IgA (detection range 0.78 ng/mL-50 ng/mL), Abcam, Cambridge, UK, interleukin (IL)-4 (detection range $31.2-2 \times 10^3$ pg/mL), interferon-gamma (IFN- γ) (detection range $15.6-1 \times 10^3$ pg/mL) and tumor necrosis factor-alpha (TNF- α) (detection range $15.6-1 \times 10^3$ pg/mL), R&D Systems, Minnesota, United States].

Cellular cytotoxicity assay

Infant peripheral blood mononuclear cells (PBMCs) were collected from the ficoll interphase. PBMCs were isolated by Human Peripheral Blood Lymphocyte Separation Kit (Tianjin Haoyang Biological Products Technology Co., Ltd.). The cytotoxicity of natural killer (NK) cells was assessed by using the CytoTox 96 kit (Promega).

Lymphocyte stimulation index test

PBMCs isolated were stimulated with phytohemagglutinin (PHA, Sigma, USA) for 72 h, shaken once a day, 50 μ L of MTT (5 mg/mL) was added to each well and they were incubated for 4 hours. The optical density (OD) values were measured at wavelengths of 570 nm and 630 nm. Results were expressed as stimulation index (SI). The equation of SI was shown as below, $SI = (\text{test well OD}_{570} - \text{test well OD}_{630}) / (\text{control well OD}_{570} - \text{control well OD}_{630})$.

Study monitoring and data quality control

All implementers were well trained. Internal monitoring of the questionnaires was performed by a panel of nutrition experts and researchers, and the investigators timely check and fill in the missing questionnaires. The operators collected, processed and detected the samples strictly following the operating procedures. Data were double entered for verification, entry and logical error detection to reduce information bias.

Statistical evaluation

The data were analyzed using SPSS 22.0 statistical software. The demographic characteristics and measurement data tested of the study population were presented as mean with standard deviations (SD) for normally distributed data or medians and interquartile ranges (IQR) for

non-normal data (continuous variables), which were compared by the unpaired Student's t test or rank sum test between the two groups. The rank sum test was used for comparison of the Igs, and the other measurement date compared by the unpaired Student's t test. The count data presented as relative numbers and proportions for categorical variables were analyzed using the χ^2 test. The p values < 0.05 were regarded as statistically statistical.

RESULTS

Questionnaire: clinical data

The clinical characteristics provided by the parents on each child were shown in Table 1. There was no significant difference in birth weight and length, age, sex, gestational age and the mode of delivery between breast-fed and formula-fed infants ($p > 0.05$). Note that all included subjects have been vaccinated with hepatitis B vaccine and Bacillus Calmette-Guerin (BCG), and vaccinated on time during the trail, as whether to get vaccinated was a potential confounding factor.

Different plasma Igs and cytokines concentrations and immune cell activity in formula-fed and breast-fed infants

At 3-month after infants enrolled, several immunological indicators of peripheral blood of infants were examined. The medians and IQR or means and SDs were presented in Table 2. The concentrations of the total IgG ($Z = -3.21$, $p = 0.001$), IgG1 ($Z = -2.12$, $p = 0.034$), IFN- γ ($t = -2.09$, $p = 0.039$) and NK cell activity ($t = -2.14$, $p = 0.034$) in the formula-fed group were higher compared with those in the breast-fed group.

However, the long-term impact of different feeding strategies remains unclear. The participants were followed for the 9-month after infants enrolled, and repeated measure was conducted. The total IgG ($Z = -4.34$, $p < 0.001$), IgA ($Z = -2.05$, $p = 0.041$) and TNF- α ($t = -2.10$, $p = 0.037$) in the breast-fed infants were lower than those in the formula-fed group, but the former had a higher lymphocyte stimulation index ($t = 2.76$, $p = 0.007$) (Table 2).

Different serum total IgG levels at different months of age in formula-fed and breast-fed infants

Only IgG is transferred from mother to fetus through the placenta. After birth, infant begins to synthetic synthesis IgG since three months, and is close to adult level when

Table 1. The clinical characteristics of two groups

	Breast-fed group (n=0)	Formula-fed group (n=100)	p value
Birth weight (kg)	3.30 \pm 0.40	3.23 \pm 0.29	0.281
Birth length (cm)	50.0 \pm 1.59	50.0 \pm 1.43	0.783
Age (d)	60.7 \pm 26.2	68.1 \pm 27.2	0.076
Sex (n)			0.541
Male	32 (45.7%)	41 (41%)	
Female	38 (54.3%)	59 (59%)	
Term delivery or not (n)			0.824
Yes	59 (84.3%)	83 (83%)	
No	11 (15.7%)	17 (17%)	
The mode of delivery (n)			0.375
Cesarean section	24 (34.3%)	41 (41%)	
Eutocia	46 (65.7%)	59 (59%)	

Table 2. Immunological indicators in formula-fed and breast-fed infants[†]

	At 3-month after infants enrolled			At 9-month after infants enrolled		
	Breast-fed group (n=70)	Formula-fed group (n=100)	<i>p</i> value	Breast-fed group (n=70)	Formula-fed group (n=100)	<i>p</i> value
Immunoglobulins						
Total IgG (g/L)	4.06 (3.33-4.73)	4.69 (3.86-5.44)	0.001	8.00 (6.37-9.38)	10.4 (8.27-12.2)	<0.001
IgA (g/L)	0.30 (0.20-0.41)	0.33 (0.21-0.52)	0.301	0.54 (0.26-0.82)	0.71 (0.26-0.98)	0.041
IgM (g/L)	0.55 (0.43-0.85)	0.63 (0.52-0.79)	0.173	1.14 (0.04-1.64)	1.42 (0.04-1.93)	0.257
IgG1 (g/L)	1.79 (0.99-3.18)	2.47 (1.15-4.51)	0.034	1.89 (1.22-2.64)	1.97 (1.42-2.67)	0.412
IgG2 (g/L)	0.61 (0.38-0.93)	0.57 (0.27-0.99)	0.810	0.88 (0.73-1.11)	0.92 (0.76-1.08)	0.810
Cytokines						
IL-4 (pg/mL)	29.3±14.3	34.9±18.1	0.061	81.2±51.0	87.5±49.2	0.519
IFN-γ (pg/mL)	55.4±22.9	65.9±23.3	0.039	69.9±39.6	81.2±33.8	0.066
IFN-γ/IL-4 ratios	2.55±1.32	3.06±1.63	0.153	1.11±0.98	1.24±0.83	0.458
TNF-α (pg/mL)	295±193	348±212	0.134	240±82	276±123	0.037
Cell viability and functional activity						
Cytotoxicity of NK cells	25.9±5.83	28.2±5.91	0.034	36.3±13.7	35.6±13.9	0.765
Lymphocyte stimulation index	1.07±0.07	1.07±0.07	0.844	1.07±0.06	1.04±0.06	0.007

[†]These immunological indicators were divided into three categories including immunoglobulins, cytokines and cell viability and functional activity. Immunoglobulins were presented as medians and IQR and analyzed using rank sum test. Cytokines, cytotoxicity of NK cells and lymphocyte stimulation index were expressed as means±SDs, and analyzed using the unpaired Student's *t* test.

Table 3. Correlation between breastfeeding and health outcomes in infants[†]

	At 3-month after infants enrolled			Difference <i>p</i> -value (2)-(3)	At 9-month after infants enrolled			Difference <i>p</i> -value (5)-(6)
	Total (1)	Breast-fed (2)	Formula-fed (3)		Total (4)	Breast-fed (5)	Formula-fed (6)	
(1) Infant had suffered from a respiratory tract infection in the past month	60	21	39	0.227	103	39	64	0.277
(2) Infant had suffered from a diarrhea in the past month	19	7	12	0.684	45	20	25	0.603
(3) Infant had suffered from a allergy or allergic reaction in the past month	2	0	2	0.513	7	2	5	0.764
N	81	28	53		155	61	94	

[†] Data were expressed as n and analyzed using the χ^2 test

grows up to 3-5 years old. Therefore, infants obtain IgG mainly through breastfeeding or formula feeding before three months old. Because our subjects were at age 10-days to 4-months before feeding, considering the possible impact of age, the concentrations of the total IgG were further analyzed at different age groups.

There was no significant difference in age distribution between breast-fed and formula-fed infants ($p>0.05$). We found that total IgG level in the formula-fed group was higher compared with those in the breast-fed group, with $Z=-2.13$, $p=0.033$ in 2-3 months of ages group at 3-month after infants enrolled. At 9-month, differences of total IgG level in two groups at ages 2-3 months and 3-4 months were $Z=-2.31$, $p=0.021$ and $Z=-3.65$, $p<0.001$, respectively (Figure 2, 3).

Formula feeding do not significantly increase diarrhea, respiratory infections and allergies in infants

When formula-fed and breast-fed infants were compared, the formula-fed group did not exhibit significantly higher levels of the diarrhea, respiratory infection or allergies or allergic reactions compared with those that were fed with breast milk ($p>0.05$) (Table 3). There were a few allergic individuals in formula and breastfeeding. During the 3-month feeding period, two individuals fed with formula suffered from a short-term skin problem, while none occurring in breastfeeding group. Infants were followed up for 9-month. In the breast-fed group, one case with aller-

gy of ampicillin (1.4%), and one case occurring short-term skin problem (1.4%). Of 100 formula-fed infants, one (1%) had allergy of amoxicillin and four (4%) had short-term skin problem.

IgG1 and IgG2 levels were lower in infants with respiratory infections compared to those uninfected

We suspect that there are differences between infections and uninfected infants of diarrhea or respiratory in those immunological indexes. No differences found in diarrhea infants and uninfected ones. However, in formula-fed group, infants without respiratory infections have higher concentration of IgG1 ($Z=-2.97$, $p=0.003$) and IgG2 ($Z=-2.09$, $p=0.037$) than infected infants at 3-month after infants enrolled (Figure 4, 5). But the differences were not statistically significant between the groups at 9-month ($p>0.05$).

DISCUSSION

The early postnatal nutrition is a vital determinant of infant growth, metabolic outcome, and long-term health,^{19,20} which includes the function of the immune system in developing neonate and even young children (12 years old).²¹ In order to evaluate the effects of two early feeding strategies on the immune function of infants, Igs, cytokines, in vitro lymphocyte proliferation assay, and cytotoxic assay were performed, which may help in the understanding of specific and non-specific immune

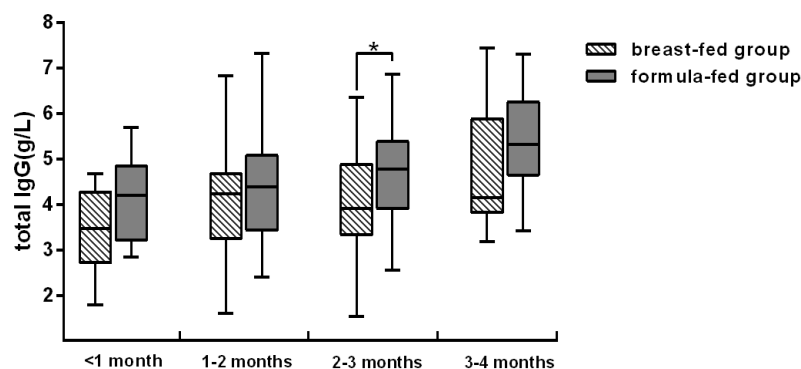


Figure 2. Serum total IgG concentrations in formula-fed and breast-fed infants at T1. <1 month infants [breast-fed (n=8); formula-fed (n=21)], 1-2 months infants [breast-fed (n=28); formula-fed (n=21)], 2-3 months infants [breast-fed (n=23); formula-fed (n=41)], 3-4 months infants [breast-fed (n=11); formula-fed (n=23)]; * $p<0.05$, ** $p<0.01$.

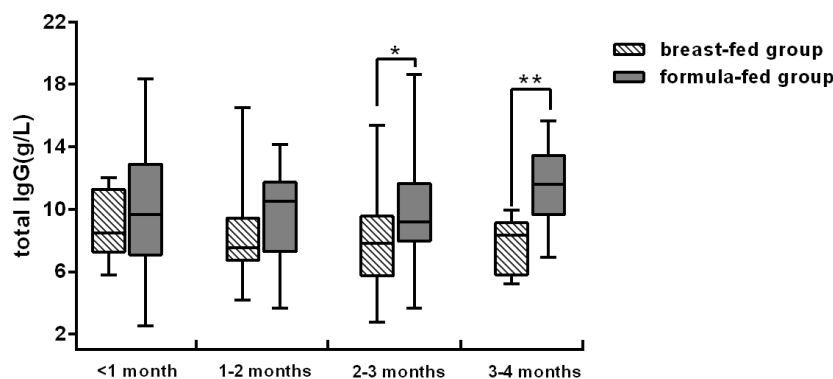


Figure 3. Serum total IgG concentrations in formula-fed and breast-fed infants at T2. <1 month infants [breast-fed (n=8); formula-fed (n=21)], 1-2 months infants [breast-fed (n=28); formula-fed (n=21)], 2-3 months infants [breast-fed (n=23); formula-fed (n=41)], 3-4 months infants [breast-fed (n=11); formula-fed (n=23)]; * $p<0.05$, ** $p<0.01$.

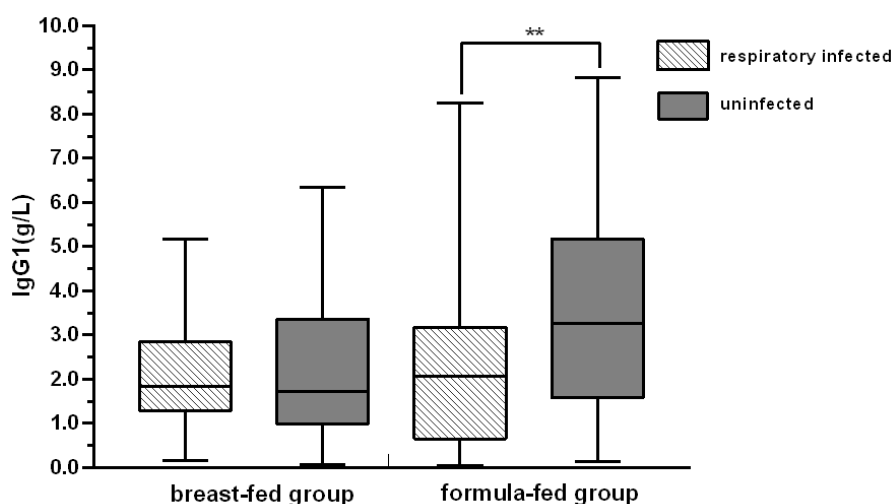


Figure 4. Comparison of IgG1 in respiratory infections and uninfected infants. Breast-fed infants (n=70), formula-fed infants (n=100). * $p < 0.01$

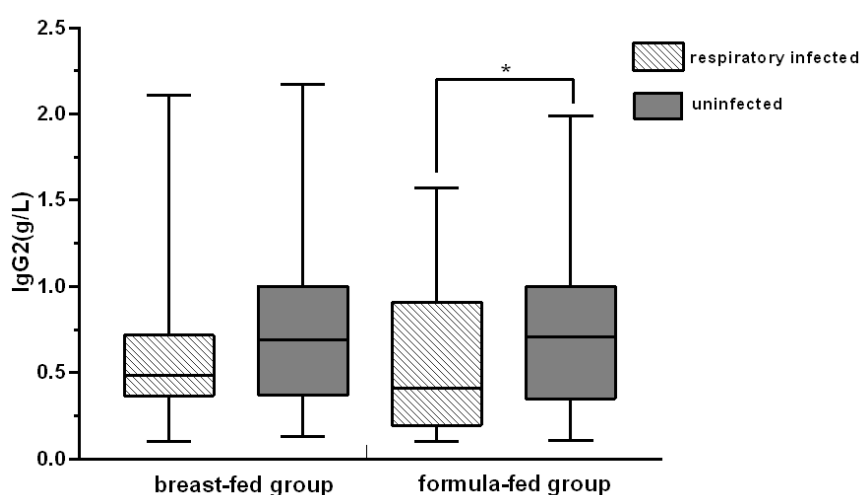


Figure 5. Comparison of IgG2 in respiratory infections and uninfected infants. Breast-fed infants (n=70), formula-fed infants (n=100). * $p < 0.05$

function.

We found that formula-fed and breast-fed have different contributions to infant immune status in the study area of Hunan. One of the most striking differences between breast-fed and formula-fed infants presented in the serum concentrations of the total IgG, IgG1, NK cell activity and IFN- γ , in which formula-fed infants exhibiting significantly higher levels of the indicators. After 9 months, concentrations of the total IgG formula-fed infants still remain higher, however these infants showed higher concentrations of TNF- α . Conferring the baby's own synthetic IgG from the third month, the results was analyzed by age groups and found that the difference also appeared under 3 months of infants, which showed that feeding might play a role in IgG. And the result was also partly caused by the babies' own synthesis. It is well known that Ig plays an important role in maintaining normal humoral immune function, notably IgG possesses anti-inflammatory properties. IgG has four subclasses in human, IgG1, IgG2, IgG3, and IgG4, which differ in their biological function. IgG1 and IgG2 were detected in our study, because of the content together can reach 90% in normal human serum. In formula-fed group, infants with

respiratory infections have lower concentration of IgG1 and IgG2 than those uninfected infants at 3-month after infants enrolled. These data suggested that IgG1 and IgG2 protect infants against respiratory infections. Furthermore, it seems that increasing total IgG might be caused by the increase of IgG1, which has protection effects against infection. The main humoral mediators of the mucosal first-line of defense system are secretory IgA and IgM. There was no significant difference discovered in infants fed after 3 months, while in the follow up period, concentration of IgA of formula-fed infants was higher than breast-fed individuals. As early as 1991, it was reported that natural killer cell percent cytotoxicity of infants at 2 months of age was significantly higher in the breast-fed compared with the standard SMA formula group.²² However in the study, formula-feeding infants resulted in higher NK cell activity than those in the breast-fed group.

There are several possible explanatory models for the observed results. We presume that the conflict results could be related to the nutritional status of lactating mothers in different areas. Our previous research in a city of south-central China found that lactation period was an important factor affecting milk composition and a dietary

pattern with high intake of red meat, cereals, and eggs was associated with higher protein, total dry matter, and energy contents in breast milk.²³ In this study, all infants came from a rural area. In order to understand the nutritional status of the lactating mother, a scientific dietary survey on the lactating mother should be conducted in this study area. Through the investigation might discover the correlation between breast-fed and infant health, as well as the existing problems, then appropriate dietary interventions could be implemented. It has been reported that malnutrition and micronutrient deficiencies are common in women of childbearing age in China, especially urban and rural lactating mothers, which may be affected by irrational food structure, food preference and hypotrophy.²⁴⁻²⁶ An investigation found that all daily food intake in the first month after delivery in 2013 was lower than that recommended by the Chinese Dietary Guidelines (2016).²⁷ The unfavorable lactating mother's nutrition status could directly lead to malnutrition and nutrient deficiency, the secretion of human milk and its trace elements (zinc, copper and magnesium) might be reduced, then will effect infants' growth and development.^{28,29} The researchers mentioned that the concentration of IgG in breast milk of healthy mothers was generally low and constant for the first 60 weeks.³⁰ A multi-centric cross-sectional study covering 8 months of lactation for 450 mothers, between October 2011 and February 2012 in three Chinese cities (Beijing, Suzhou and Guangzhou), demonstrated that serum albumin and IgG levels appeared stable throughout lactation.³¹

But there were no related studies for IgG of human milk in malnourished mothers of rural area. We suggest that malnourished mothers could might secrete IgG at reduced amount, thereby reducing the concentration of IgG in the baby's serum. In addition, it is reported that malnourished mothers may stimulate inflammation in breast-fed children.³² In addition, manipulating the composition of formula, by reducing protein content, adding prebiotics, growth factors or SIgA can modulate intestinal and pancreatic function development, and thereby may reduce the differential responses between breast-fed and formula-fed neonates.³³ It is worth mentioning that the formula milks used by majority infants contain fructooligosaccharides or alpha-lactalbumin. Fructooligosaccharides are usually added in formulas as a kind of prebiotic to make them like breast milk more.^{34,35} Alpha-lactalbumin appears to be fully hydrolysed and absorbed in the infant intestine, thus to be a good source of nitrogen and indispensable amino acids.³⁶ In line with this, Bruck et al observed inhibition of *E. coli*-induced diarrhoea in infant monkeys fed a formula supplemented with bovine α -lactalbumin.³⁷ These results could might be helpful to understand that early formula feeding did not significantly increase infant diarrhea and respiratory infections in our study.

The results of the contrast of IgA in breast-fed and formula-fed infants were conflicting in the previous literature, closely related to infants' age.³⁸⁻⁴⁰ The concentrations of salivary IgA over the first 6 months of life were significantly higher in formula- compared to breast-fed infants.^{38,40} And it is reported that at 6 months of age, breast-fed infants have significantly increased IgA, com-

pared to formula-fed infants.⁴⁰ Our study was similar to the reports of Avanzini and Gross & Buckley, which indicated that serum IgA is higher in formula-fed infants than breast-fed infants but with no significant difference after feeding for 3 months. This obvious difference could be related to age distribution of infants, since there was 15.7% in breast-fed group and 23% in formula-fed group infants' ages over 6 months at first follow-up time. While in the followed-up, the difference was statistically significant. It has been demonstrated that serum IgA and IgM may develop during the early stage of life.⁴¹ Since infants begin to fed, formulas are more likely to be antigens from the external environment compared with breast milk, a cross-talk between the intestine (flora colonization) and environmental antigens could might increase the concentrations of serum IgA. And secretory IgA (SIgA) is thought to be critical in the infant gut as it neutralizes bacterial and viral pathogens by binding to them, thus reducing their ability to interact with epithelial cells and infect.⁴²

In our study, no significant difference of allergy was found between the groups. Early dietary intake may influence the development of allergic diseases.⁴³ None have reached a definitive conclusion as to whether breastfeeding is an effective strategy to prevent allergic diseases, even different population studies remain contradictory.⁴⁴⁻⁴⁸ Allergies are understood to be typical diseases with an elevation of helper T (Th) 2 cytokines, also related to IFN- γ secreted by Th1 cells.⁴⁹ Investigating typical Th1 cytokines (such as IFN- γ and TNF- α) as well as the typical Th2 cytokine IL-4 might be valuable. We found that infants who were formula-fed for 3 months showed increased serum levels of IFN- γ when compared to those who received breast milk, it was consistent with Winkler B's findings,²¹ and with higher TNF- α at 9-month after infants enrolled. But there was no statistical difference in IFN- γ /IL4 ratio between two groups.

Above all, this was the first report about different effects of formula feeding and breastfeeding in Hunan province. Because the blood specimens of infants are difficult to obtain, the previous few experimental studies only reported fewer subjects. Compared with them, the sample size of this experiment might be greater. Additionally, comprehensive detection targets were designed including Igs, cytokine productions, cytotoxicity of NK cells and lymphocyte stimulation index, and aimed to characterize the differences of immune physiology in breast-fed and formula-fed infants. However, there were several limitations to this work. First, the main limitation of this study is that we did not conduct a dietary survey during the feeding, designed to prospectively access local mothers' food consumption, energy and nutrient intake and related chemical contaminant exposure to explore the relationship of diet with health. Second, these experiments should be carried out as early as the initial feeding to know the baseline levels which might affect interpretation of experimental results and conclusions.

Conclusions

This finding suggests that formula-fed and breast-fed have different contributions to infant immune development depending on the results. There was no obvious

association of increase risk of infantile diarrhea and respiratory infections between breastfeeding and formula feeding. Although breast milk is the ideal source of nutrition for infants, in the case of poor health or nutritional status of the lactating mother, infant formula could be appropriately added.

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AUTHOR DISCLOSURES

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