

Immunomodulatory constituents of human milk change in response to infant bronchiolitis

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Although epidemiological evidence is generally supportive of a causal association between respiratory syncytial virus (RSV) bronchiolitis during infancy and the development of persistent wheeze/asthma, if not allergy, the mechanism by which this occurs and an explanation for why all children do not succumb remains to be elucidated. Breast feeding has been found to confer a protective effect against respiratory infections such as RSV bronchiolitis and allergy; however, again there is little direct evidence and no clear mechanism. In this study, we examined whether human milk immunomodulatory factors (cells, cytokines) change in response to clinically diagnosed, severe bronchiolitis in the recipient breast-fed infant. We examined milk from 36 breast feeding mothers of infants hospitalized with bronchiolitis and compared them with milk from 63 mothers of postpartum age-matched healthy controls. Milks from mothers of infants hospitalized with bronchiolitis had significantly greater numbers of viable cells when compared with the milks obtained from mothers of healthy infants (1.3 ± 0.4 vs. $0.3 \pm 0.03 \times 10^6$ cells/ml, mean $\pm$ s.e.m.; $p \leq 0.001$). Further, the cells obtained from the mothers of infants hospitalized with bronchiolitis were found to produce a skewed cytokine profile *ex vivo* in response to stimulation by live RSV but not when cultured with a non-specific mitogen (concanavalin A). This study provides preliminary evidence for an immunological link between mothers and their breast-fed infants during severe respiratory infections as well as a possible contributing factor to the development of persistent wheeze in these infants.

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The majority of infants encounter respiratory syncytial virus (RSV) during the first 12 months of life, often manifesting as severe bronchiolitis requiring hospitalization. Epidemiological studies demonstrate a link between RSV bronchiolitis in infants and the development of persistent wheeze or asthma during childhood (1, 2). However, the mechanism by which this occurs, as well as an explanation for why all infants contracting RSV bronchiolitis do not progress to asthma has yet to be elucidated.

Activation of the immune system by infections such as RSV significantly alters the cytokine

profile produced by immunoactive cells. These changes affect cells not only at sites of activation, such as the respiratory mucosal surfaces, but also systemically in peripheral blood. The peripheral blood mononuclear cells (PBMC) of infants hospitalized with RSV-mediated respiratory tract infections demonstrate a significantly elevated interleukin (IL)-4 to interferon (IFN)- γ ratio upon mitogen stimulation *in vitro* (3). Similarly, the PBMC of atopic adults produce higher levels of T helper (Th) 2 cytokines, IL-4 and IL-5, and reduced levels of Th1 cytokines, IL-12 and IFN- γ (3, 4), demonstrating a Th2 bias. This Th2 bias,

as well as increases in chemokines are also found in the aqueous fraction of human milk from atopic mothers (4–8).

There is some debate about the protective effect of breast feeding against respiratory infections such as RSV-mediated bronchiolitis. Although the epidemiological evidence is generally supportive of this concept there are few specific examples of protection and the potential underlying mechanisms are unclear (9). Human milk provides protection for the recipient infant by the transfer of a number of factors including anti-infectious agents such as immunoglobulins, lactoferrin and oligosaccharides (7). However, it may be speculated that long-term benefits must be derived from human milk factors capable of modulating the developing infant immune system. A number of potentially immunomodulating factors have been described in human milk, including many cytokines and maternally derived human milk cells.

Cells derived from human milk produce a number of cytokines *ex vivo* both constitutively and after stimulation with mitogens (10, 11). As viable human milk cells have been isolated from the stools of human milk-fed infants, it can be postulated that the immature digestive system of the recipient infant does not digest or disable a substantial proportion of these cells (12). While the source of human milk cells has not been adequately identified, there is evidence that at least some of the cells have homed directly from mucosal sites such as the gastrointestinal tract following *in situ* activation (13, 14). As the cytokines known to be affected by RSV infection are also produced by human milk cells, it is reasonable to postulate that these effects may be conferred on the cells delivered to the infant via breast feeding.

In this study, we examined whether human milk cells and cytokines change in response to clinically diagnosed, severe bronchiolitis in the breast-fed infant.

Materials and methods

Subjects

Thirty-six breast feeding (>80% milk feeds as breast milk) mothers of term infants (≥37 wk of gestation) provided a single breast milk sample while their infant was hospitalized with bronchiolitis. Clinical diagnosis of bronchiolitis was defined as wheezing illness in an infant (≤12 months of age) caused by a viral respiratory tract infection associated with hyperinflation. Each infant admitted with bronchiolitis underwent a routine nasopharyngeal aspirate (NPA)

for viral identification. A separate group of 63 postpartum age-matched women who had a healthy breast feeding child also provided a breast milk sample. A series of questions were asked relating to socio-economic status, number of children in the household, exclusivity of breast feeding and consumption of any alcohol or cigarettes (yes/no). Socio-economic status was defined through two parameters. The first being a scale of highest level of education achieved with a score of 1 being the lowest (less than or equal to year 11 of secondary school) and 6 being the highest (postgraduate degree). The second measure ranked occupation according to the scale developed by Daniel (15) which assigns a score based on power, privilege and prestige of occupations in Australia ranging from 1 as the highest level (e.g. 1.2 Judge) to 6 as the lowest level (e.g. 6.0 unemployed). Data relating to maternal illness (yes/no) and self-reported atopic status [allergic rhinoconjunctivitis, atopic asthma, atopic dermatitis or food allergy and as well as total serum immunoglobulin (Ig) E; Imx Total IgE Kit; Abbott Laboratories, Abbott Park, IL, USA] were used to determine maternal atopic status. All samples were collected with the approval of the Flinders Clinical Research Ethics Committee of Flinders Medical Centre, South Australia and with the informed consent of each of the participants.

Collection and processing of human milk samples

Mothers were asked to provide a complete expression from one breast at least 2 h after the last feed from that breast, to minimize intra-feed variation and during the morning to limit diurnal variation. Samples were expressed using an electric pump (Ameda, Hollister, Chicago, IL, USA) which collects directly into a sterile infant feeding bottle. The sample was transferred into sterile polypropylene centrifuge tubes (Greiner Labortechnik GmbH, Frickenhausen, Germany) and centrifuged at $890 \times g$ for 30 min before the fat layer was removed. The aqueous fraction was collected, aliquoted and stored at -80°C until analysis by enzyme-linked immunosorbent assay (ELISA).

Human milk cell culture

The resulting cell pellet was resuspended and transferred to a clean tube in phosphate-buffered saline, washed $\times 1$, the supernatant discarded and the final pellet resuspended in sterile RPMI-1640 (2 mM L-glutamine, 10 mM HEPES, 50 U/ml penicillin, 37.5 U/ml streptomycin, 10%

endotoxin-free, heat-inactivated foetal bovine serum; CSL Biosciences, Parkville, Australia). An aliquot was taken for determination of total viable cell number by direct microscopy with trypan blue staining.

Total human milk cells (2.5×10^6 cells/ml) were cultured in sterile 96-well flat bottom cell culture plates (Greiner Labortechnik GmbH) in the presence or absence of $150 \mu\text{g/ml}$ concanavalin A (ConA; Sigma Chemical Company, St Louis, MO, USA) at 37°C , 5% CO_2 . After 48 h, cells were lysed by a single freeze-thaw cycle (-80°C) and the medium harvested and centrifuged at $890 \times g$ for 5 min to pellet cell debris. Supernatants were collected, aliquoted and stored at -80°C until analysis by ELISA.

Respiratory syncytial virus propagation

The RSV used for this study was characterized as the long strain of RSV (88:RS4; Dr P. Young, Sir Albert Sakzewski Virus Research Centre, Queensland, Australia). The virus was grown in confluent monolayers of A549 cells as described previously (16). Stock virus was between 1 and 3×10^8 fluorescent focus units (ffu)/ml. UV-inactivated RSV and A549 conditioned media controls were obtained as previously described (16).

Stimulation of human milk cells with RSV

Provided sufficient cells were obtained, total human milk cells (10×10^6 cells/ml) were cultured in the presence or absence of live RSV at a multiplicity of infection of 3. Aliquots were also treated with UV-inactivated virus or A549 conditioned medium. After 1 h the supernatant was carefully removed to discard any unbound virus and $200 \mu\text{l}$ of RPMI added. After 24 h, cells were lysed, medium harvested and centrifuged and supernatants were collected, aliquoted and stored at -80°C until analysis by ELISA.

Flow cytometry of human milk cells

In the event of cells remaining after preparation of the cell culture assay, cells (1×10^6 cells) were stained with fluorochrome-labelled monoclonal antibodies for the determination of leucocyte subsets by 2-colour direct immunofluorescence on a FACScan instrument (Becton Dickinson, San Jose, CA, USA). Aliquots of cells were labelled with directly conjugated mouse monoclonal IgG antibody to CD45 (leucocyte common antigen) phycoerythrin (PE)-Cy 5 and directly conjugated mouse monoclonal IgG anti-

body to either CD14, or a combination of CD3/CD19; CD3/CD4; CD3/CD8 (PE or fluorescent isothiocyanate; Becton Dickinson). Each sample was live gated using CD45 fluorescence and side-scatter parameters to collect 10,000 CD45-positive events.

Immunoassay

Interferon- γ , IL-2, regulation on activation normal T cell expressed and secreted (RANTES; R&D Systems Inc., Minneapolis, MN, USA) IL-4, IL-10 (Endogen, Woburn, MA, USA) and IL-8 (BD Pharmingen, San Diego, CA, USA) were measured in the cell culture supernatants by ELISA, using matched antibody pairs at concentrations recommended by the manufacturer, as described previously (16). The minimum detectable level for each assay was 8, 8, 4, 5, 5 and 20 pg/ml, respectively.

Statistical analysis

The data obtained were initially analysed as a matched case-controlled study using conditional logistic regression to determine the parameters associated with infant bronchiolitis. The conditional logistic regression analysis was performed using STATA for Windows 8.0 (StataCorp LP, TX, College Station, USA). Cytokine levels obtained are expressed in pg/ml (median; percentiles and outliers). Samples below the minimum detectable level were assigned a value of half that level for statistical analysis. Cytokine data were not normally distributed and could not be corrected via log-transformation and were therefore analysed using Mann-Whitney U and Kruskal-Wallis non-parametric tests, each with exact Monte Carlo correction. Subject and sample characteristics as well as flow cytometry data were examined via independent samples t -test. Cell types are given as percentage total leucocytes or lymphocytes as appropriate (mean $\pm$ s.e.m.). Data were normalized by log-transformation as required. A probability level of ≤ 0.05 was considered statistically significant and all analyses were performed using SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, USA) unless otherwise stated.

Results

Subject characteristics

Mothers of bronchiolitic infants were younger, of lower socio-economic status and had larger families than the mothers in the control group.

Table 1. Characteristics of lactating women according to enrolment group as defined by clinical category of their infant [mean $\pm$ s.e.m. (range) or percentage (affirmatives/group size), as indicated]

	Bronchiolitic group (n = 36)	Healthy group (n = 63)
Age (yr)	29 $\pm$ 1 (18–38)*	32 $\pm$ 1 (21–42)
Occupation	4.8 $\pm$ 0.2 (1.8–5.9)*	4.3 $\pm$ 0.1 (1.9–5.9)
Education	2 $\pm$ 0 (1–6)*	4 $\pm$ 0 (1–6)
Smoking	22% (8/36)	6% (4/63)
Alcohol	39% (14/36)	44% (28/63)
Postpartum age (days)	121 $\pm$ 14 (13–322)	140 $\pm$ 10 (15–330)
Children	3 $\pm$ 0 (1–11)*	2 $\pm$ 0 (1–6)
Infant RSV-positive NPA	53% (19/36)	Not tested
Illness†	69% (25/36)*	44% (28/63)
Mastitis	3% (1/36)	3% (2/63)
Atopy	72% (26/36)	81% (51/63)
Asthma	33% (12/36)	33% (21/63)
Hayfever	50% (18/36)	59% (37/63)
Dermatitis	28% (10/36)	37% (23/63)
Food allergy	6% (2/36)	17% (11/63)
Total serum IgE (IU/ml)	60 $\pm$ 16 (2–462)	98 $\pm$ 52 (1–3030)

*p $\leq$ 0.05.

†Maternal illness was recorded based on symptoms suffered during the week prior to sample collection reported by the individual, not on clinical diagnosis. Symptoms reported were limited to mild to moderate upper respiratory tract infection.

RSV, respiratory syncytial virus; NPA, nasopharyngeal aspirate; Ig, immunoglobulin.

Not unexpectedly, a significant difference was found in the rate of illness between the enrolment groups, with the mothers of bronchiolitic infants reporting a greater incidence of illness themselves than the control mothers (Table 1).

Fifty-three percentage of the bronchiolitic infants whose mothers were enrolled in the study returned a NPA result positive for RSV. Only one of the remaining 17 infants gave a sample positive for another respiratory virus (Parainfluenza virus 3). Twelve of the infant NPA samples were negative for all respiratory viruses examined. NPA results were unable to be obtained for the remaining four infants.

Sample collection

Samples collected from the enrolment groups were similar for both collection time and volume. However, they did differ in the amount of time since the last feed from the breast from which the sample was collected (Table 2).

Human milk cell composition and activation

A significant difference was found in the total number of cells per millilitre of milk between mothers of infants with bronchiolitis and healthy controls (Table 2). There was no difference in cell number between the mothers of those infants with confirmed RSV-mediated bronchiolitis and bronchiolitis where no specific virus could be identified (data not shown).

There was no significant difference between the groups in the cell types present when considering both the major cell types of monocytes, lymphocytes and granulocytes (Table 2) as well as the lymphocyte subsets of B cells, T cells, T helper/inducer (CD4⁺) cells and T cytotoxic/suppressor (CD8⁺) cells (Table 2).

Human milk aqueous fraction cytokines

There was significantly less IL-10 in the milks of mothers of infants with bronchiolitis when compared with milks of the mothers of healthy infants (Fig. 1b) and was more pronounced when mothers of RSV-positive infants were examined separately from mothers of negative bronchiolitic infants [7, 2.5–167 (RSV-positive) vs. 59, 2.5–2053 (virus-negative) vs. 63, 2.5–751 (healthy control); median, 10–90th percentiles].

There was no significant difference in the amount of any other cytokine tested between the milks from the mothers of bronchiolitic

Table 2. Sample characteristics according to enrolment group [mean $\pm$ s.e.m. (range)]

	Bronchiolitic group (n = 36)	Healthy group (n = 63)
Collection time (am)	10:26 $\pm$ 0:09 (8:00–12:00)	10:24 $\pm$ 0:06 (8:30–12:30)
Time since last feed (min)	169 $\pm$ 18 (60–540)*	221 $\pm$ 17 (60–720)
Volume (ml)	66 $\pm$ 6 (8–143)	76 $\pm$ 5 (10–148)
Total cell number/ml	1.3 $\pm$ 0.4 (0.04–15.4) $\times$ 10 ⁶ *	0.3 $\pm$ 0.03 (0.02–1.3) $\times$ 10 ⁶
Monocytes/macrophages† (% total leucocytes)	25 $\pm$ 4 (7–68)	23 $\pm$ 2 (7–52)
Lymphocytes† (% total leucocytes)	26 $\pm$ 4 (2–55)	21 $\pm$ 4 (2–62)
Granulocytes† (% total leucocytes)	48 $\pm$ 5 (17–91)	56 $\pm$ 5 (18–86)
B lymphocytes‡ (% total lymphocytes)	2 $\pm$ 0.4 (0–5)	1 $\pm$ 0.6 (0–8)
T lymphocytes‡ (% total lymphocytes)	80 $\pm$ 3 (53–96)	79 $\pm$ 3 (56–94)
CD4 ⁺ T (helper/inducer)‡ (% total lymphocytes)	48 $\pm$ 3 (28–71)	51 $\pm$ 3 (34–73)
CD8 ⁺ T (suppressor/cytotoxic)‡ (% total lymphocytes)	32 $\pm$ 3 (13–61)	32 $\pm$ 4 (10–50)

*p $\leq$ 0.05.

†Bronchiolitic group (n = 22), control group (n = 21).

‡Bronchiolitic group (n = 20), control group (n = 13).

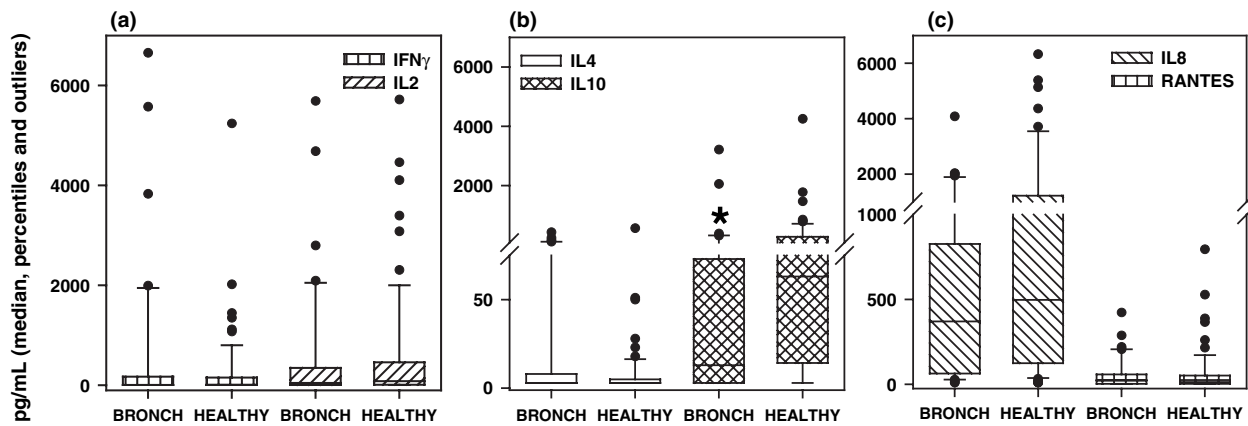


Fig. 1. Comparison of human milk aqueous phase cytokines (pg/ml) between mothers of bronchiolitic infants (BRONCH, $n = 36$) and healthy controls (HEALTHY, $n = 63$). (a) Th1 cytokines interferon- γ and interleukin (IL)-2, (b) Th2 cytokines IL-4 and IL-10 and (c) chemokines IL-8 and RANTES. Median 10th, 25th, 75th and 90th percentiles plus outliers (•). Significant differences ($p \leq 0.05$) distinguished by an *.

infants when compared with those of healthy controls (Fig. 1a–c).

Human milk cell cytokine production

Stimulation with ConA produced detectable quantities of each of the cytokines IFN- γ , IL-2, IL-4, IL-10 and RANTES, significantly increased over the constitutive production for each cytokine (data not shown, $p \leq 0.05$). This was not the case for IL-8.

Similarly, co-culture with live RSV produced detectable concentrations of each of the cytokines IFN- γ , IL-2, IL-10, IL-8 and RANTES at levels significantly elevated when compared with that of controls. IL-4 was not produced at detectable levels in response to stimulation with live RSV in any sample. Culture with live RSV increased the production of IFN- γ and RANTES above the level attained in response to UV-inactivated RSV. However, the production of IL-2, IL-10 and IL-8 was induced to the same extent by both live and UV-inactivated RSV (data not shown, $p \leq 0.05$).

Constitutive production of IFN- γ by cells obtained from mothers of bronchiolitic infants was significantly less than that from the mothers of healthy infants over a 24-h incubation [4 pg/ml, 4–31 (BRONCH) vs. 14 pg/ml, 4–43 (HEALTHY); median, 10–90th percentiles]. This decreased production was maintained with RSV stimulation of cells (Fig. 2a–c) and resulted in an overall elevation in the Th2 to Th1 ratio of IL-10:IFN- γ (Fig. 2d). This effect was only apparent with stimulation of milk cells by RSV and not with the non-specific mitogen ConA (Fig. 3a,b). Stimulation by ConA did, however, demonstrate an increased production of RANTES by cells obtained from mothers of bronchiolitic infants

when compared with mothers of healthy infants (Fig. 3c).

Statistical analyses

The conditional logistic regression used to analyse these data leads to a model with two predictors or explanatory variables. Maternal age [odds ratio (OR) 0.87; 95% confidence interval (CI): 0.76–0.99; $p = 0.04$] and cell number ($/10^6$ cells/ml; OR 1.32; CI: 1.12–1.56; $p = 0.001$) were the only significant variables in differentiating mothers of bronchiolitic infants from those of healthy infants. Designation into each of these groups using this model, was not effected by any other subject or sample characteristics, including self-reported maternal illness or length of time since the previous feed.

The elevation of the total cell population in a mother's milk and severe respiratory illness in her infant was found to be significantly associated using both direct statistical comparison and the more rigorous logistical regression analysis.

Discussion

This study suggests two effects of clinically diagnosed disease in infants on cells in human milk, namely higher cell numbers and an alteration in the production of cellular cytokines. Interestingly, while higher cell numbers are found in human milk of mothers of infants with bronchiolitis, there is no significant difference in the proportions of each of the predominant cell types between such mothers and control mothers. Differences do exist, however, as cells from milks of mothers of bronchiolitic infants produce a significantly elevated IL-10:IFN- γ cytokine

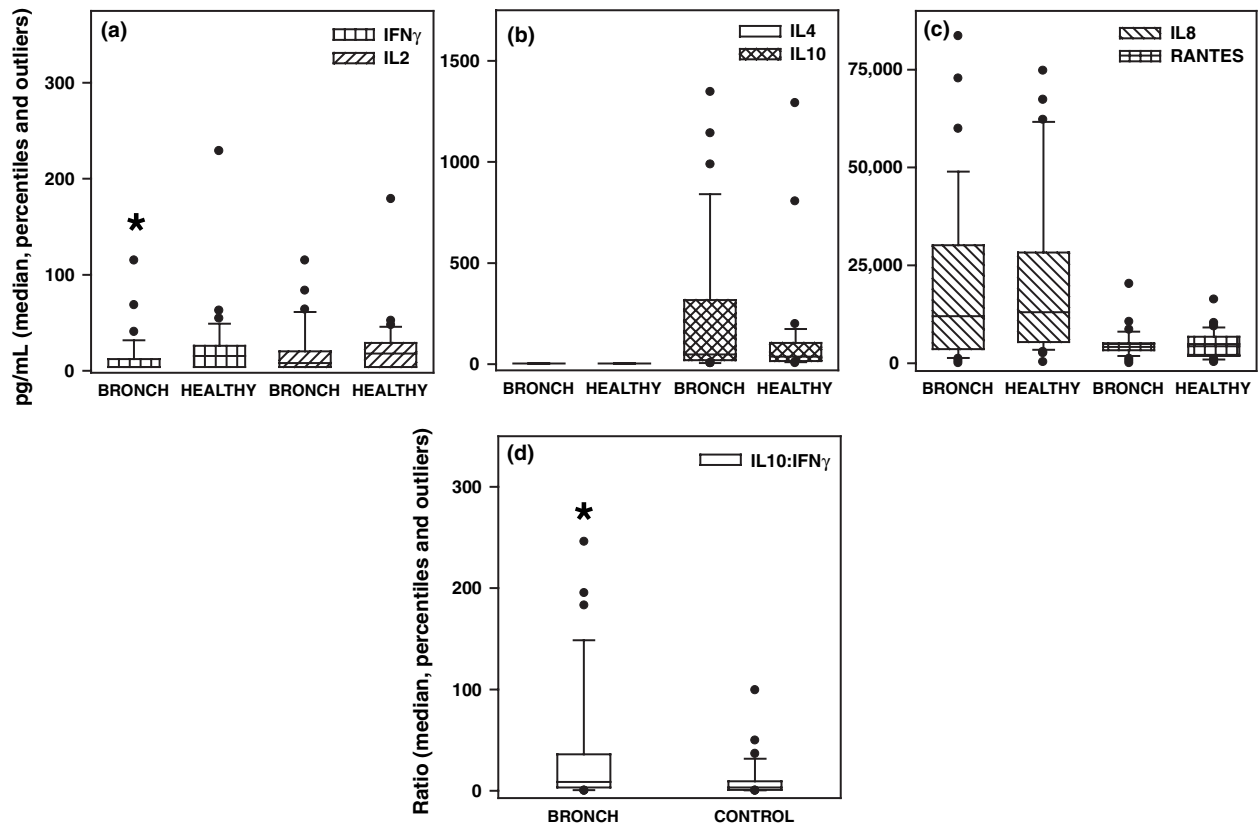


Fig. 2. Comparison of cytokines (pg/ml) produced by human milk cells stimulated with live respiratory syncytial virus [multiplicity of infection (MOI) = 3, 24 h] between mothers of bronchiolitic infants (BRONCH, $n = 33$) and healthy controls (HEALTHY, $n = 31$). (a) Th1, interferon (IFN)- γ and interleukin (IL)-2, (b) Th2, IL-4 and IL-10, and (c) chemokines, IL-8 and RANTES, (d) Th2 to Th1 ratio, IL-10:IFN- γ . Median 10th, 25th, 75th and 90th percentiles plus outliers ($\bullet$). Significant differences ($p \leq 0.05$) distinguished by an *.

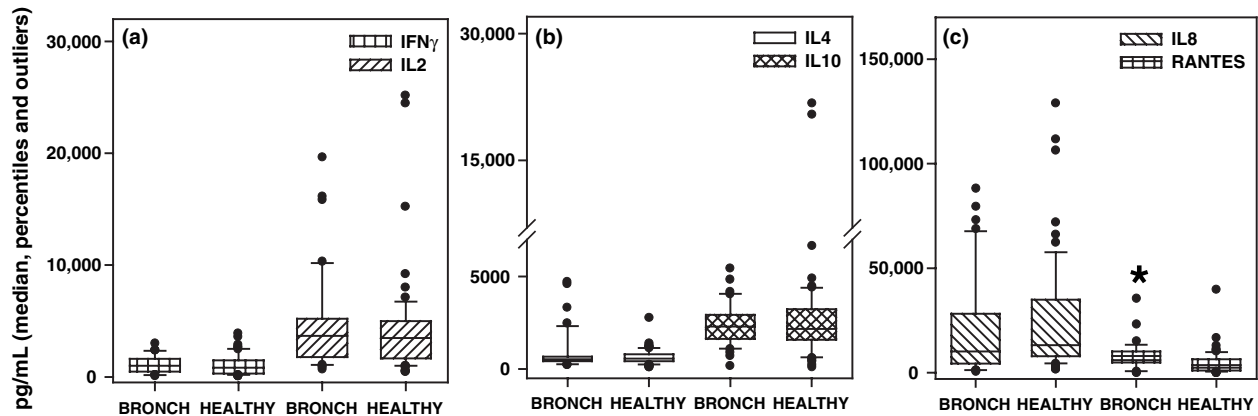


Fig. 3. Comparison of cytokines (pg/ml) produced by human milk cells stimulated with concanavalin A (150 μ g/ml, 48 h) between mothers of bronchiolitic infants (BRONCH, $n = 36$) and healthy controls (HEALTHY, $n = 61$). (a) Th1, interferon- γ and interleukin (IL)-2, (b) Th2, IL-4 and IL-10 and (c) chemokines, IL-8 and RANTES. Median 10th, 25th, 75th and 90th percentiles plus outliers ($\bullet$). Significant differences ($p \leq 0.05$) distinguished by an *.

profile. In addition, this effect is only found with live RSV, the most common causal agent for infant bronchitis, not in response to non-specific activation of the cell population, as stimulation with the mitogen ConA did not result in a similar

IL-10:IFN- γ response. These results suggest that there is activation of human milk cells from this group of mothers of infants with bronchiolitis, but that this activation differs in response to RSV in those mothers currently exposed.

Interpretation, as indicated statistically by both logistic regression and direct comparison of the two groups, while indicating a more substantial influence of infant illness as opposed to maternal, may be counterintuitive and therefore requires further investigation. The two groups of mothers varied in several regards (Table 1), i.e. socio-economic status, number of children in the household, and most important of all, in a significantly greater incidence of illness (symptoms of mild to moderate upper respiratory tract infection). Thus, the question remains open whether the composition of human milk with regard to immunological compounds rather reflects the immune status of the mother than being a reaction on the infant's bronchiolitis.

Respiratory syncytial virus is highly contagious with substantial amounts of live virus being shed by infected infants throughout the course of the disease (17). The close contact that occurs between a mother and her breast-fed infant would therefore result in substantial cross-infection. While the disease symptoms produced by RSV infection in the majority of adults are extremely minor due to the endemic nature of the virus and therefore almost complete population exposure by early childhood (18), secondary exposure to the virus via their infant may still elicit an immunological response in the breast feeding mother. This potentially subclinical infection may result in the influx of activated leucocytes into the mammary gland from where they are passed on to the infant through the milk and where secondary exposure to RSV may elicit an altered cytokine response as demonstrated *ex vivo* in this study. While survival and transfer of activated milk cells into the system of infants has been previously reported (12, 19, 20) these studies primarily relate to the immediate postnatal period. The potential for immunological action by maternally derived milk cells in infants with RSV, for which the median age is approximately 4 months, remains to be investigated. However, the age of RSV infection and bronchiolitis is as early as 2 wk postpartum allowing for speculation for a significant role of maternally derived cells, if only in these very young infants.

Th2:Th1 bias has been previously reported in peripheral blood cells from RSV-infected individuals (9) indicating that these human milk cells have homed to the mammary gland via the peripheral blood following activation by the virus within the mucosa-associated lymphoid tissue in the respiratory tract of the mother. This memory response implies that not only are the cells of breast feeding mothers of infants with bronchiolitis immunologically activated, but that

this activation, will be skewed towards the decreased production of Th1 cytokines upon secondary exposure to the virus in the recipient infant. A recent study by Koopman et al. (21) highlights the potential significance of this via a significant correlation between the development of wheeze during the first year of life with an increased serum IL-10 to IL-12 ratio.

A significant increase in RANTES in response to stimulation with ConA by cells from the milks of mothers of bronchiolitic infants may be further indicative of enhanced inflammatory activation of these cells. Enhanced production of this chemokine would hypothetically lead to an increase in the number of T lymphocytes migrating into the mammary gland. However this was not found, indicating an alternative mechanism mediating the influx of large number of cells apparently able to recognize RSV antigen and produce different levels of T-helper cytokines, without affecting the proportion of each of the major cell types found in human milk. These cells are therefore primed or activated towards a significant alteration of their cytokine production in response to the current pathogen.

The lower concentration of IL-10 found in the aqueous fraction of milks from mothers of bronchiolitic infants was unexpected as IL-10 production by alveolar macrophages and PBMCs is elevated in response to infection with RSV both *in vitro* when compared with other respiratory viruses, and *in vivo* when compared with healthy controls (22). If human milk cytokines are secreted into the milk by *in situ* leucocytes, it would therefore be expected that the levels of IL-10 would be elevated in mothers exposed to RSV via their infant rather than decreased as found in this study. Regardless of cause, the decrease in soluble IL-10 in human milk may benefit infected infants as high levels of IL-10 during RSV bronchiolitis are associated with the development of recurrent wheeze and asthma during the months following recovery (23).

The association of decreasing maternal age with bronchiolitis may be seen as a potential confounding factor in the conditional logistic regression analysis. Maternal age cannot be discounted from acting as a potential contributor to either the difference in cell number observed between the groups, or in the incidence of bronchiolitis in infants. Similarly, the difference in time since the last feed between the groups may be indicative of changes in feeding pattern of sick infants. Sick infants may take smaller volumes, more regularly which could alter the process of cellular accumulation in the breast.

No data are available which directly addresses this issue. However, there was no difference found in the volume of milk obtained from complete expressions in each group indicating little effect of infant illness on total milk volume.

By selecting infants hospitalized with a specific, clinically diagnosed condition, we have been able to identify a highly significant change in the milk that is being received by the infant who is ill as opposed to a healthy infant. This research provides some evidence for a link between the cells found in human milk and illness in the recipient infant. The significant alteration in the cytokines produced by these cells in response to secondary exposure to RSV also suggests a specific immunological outcome resultant from maternal viral exposure. This work may contribute some explanation for the inconsistent reports of protection by human milk feeding against both RSV bronchiolitis and the development of asthma.

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