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IgA Antibodies, TGF-β1 and -β2, and Soluble CD14 in the Colostrum and Development of Atopy by Age 4

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ABSTRACT

Specific defense factors in breast milk together with length of breast-feeding and genetic predisposition may modulate the development of allergy. We studied whether IgA, soluble CD14 (sCD14), or transforming growth factor (TGF)-β in colostrum could affect the development of atopy in children up to age 4. From a cohort of 4676, we selected four groups of children with either long or short exclusive breast-feeding (>3.5 or <0.5 mo); these groups further differed in the presence or absence of atopic heredity. In colostrum from mothers, we measured total IgA, IgA antibodies to cow's milk (CM) and casein, sCD14, and TGF-β1 and -β2. The children were divided into three groups: those with no atopic symptoms or IgE, those with allergic symptoms, and those with both outcomes. Mothers of infants later showing atopic symptoms or, in addition, having IgE sensitization (verified atopy) had a lower concentration of IgA casein antibodies in their colostrum than did mothers of infants with no indication of atopy at age 4. Low concentration of IgA casein antibodies was a significant risk for verified atopy. sCD14 levels were lower in colostrum of mothers with infants developing atopic symptoms and IgE sensitization than of those of infants with no atopy. Specific IgA antibodies to CM antigens and sCD14 in colostrum significantly associated with the appearance of both symptomatic and verified atopy by age 4. **(*Pediatr Res* 58: 1300–1305, 2005)**

Abbreviations

CM, cow's milk
CMA, cow's milk allergy
sCD14, soluble CD14
TGF, transforming growth factor

Interaction between early infant feeding and subsequent development of allergies has been debated for decades (1–5). Human breast milk contains antibodies to antigens the mother has come into contact with, particularly against food antigens and enteral microbes (6–10). These antibodies, together with a small amount of food antigens in the milk, may direct the immune response of the infant to develop tolerance or hypersensitivity to those antigens. Breast milk also contains a number of nonspecific anti-infectious substances such as iron-binding lactoferrin, bacteriocidic lysozyme, and the oligosaccharides inhibiting microbial attachment to epithelial cells. It therefore effectively reduces infections of the newborn infant and has an impact on infants' indigenous bacterial flora (8–10). Human milk, furthermore, contains a number of cytokines (11–16) and other immunomodulatory (17) agents as well as living cells (18), which in the immature intestine of the newborn may modulate the development of local immune responses.

We recently showed that the effect of breast-feeding on the appearance of allergic diseases at age 4 y depended on heredity: if the infant has the genetic component for allergy, long breast-feeding seems to protect against allergies; if the heredity is negative, long breast-feeding promotes emergence of allergies (5). Because of such interactions and the fact that maternal heredity has a stronger impact on the appearance of allergies than does paternal allergy (19), breast milk of mothers with allergy has been speculated to differ from that of nonallergic mothers and, in fact, some differences have emerged (20,21). Further, very little is known of the appearance of diseases during infancy and early childhood in relation to breast milk qualities (13,22).

In the present study, we aimed to determine the association of soluble factors in colostrum and the development of atopy in a large group of full-term infants, whose health we followed to age 4 and studied their allergy in detail at that age (5). We analyzed the association of IgE sensitization and allergies at

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4 y of age with breast milk–derived immunologic factors, which have been associated with the development of allergic diseases of children (total IgA, CM-specific IgA, TGF-β1 and -β2, and sCD14) (6,7,13,15–17,20–22) and took into consideration both the length of breast feeding and atopic heredity.

METHODS

Study population. The present study used the findings of our previous study (5), which collected four groups of 4-y-old children from an unselected birth cohort of 4674 infants born between August 1994 and November 1995. The children had been followed prospectively during their first year of life for the appearance of CMA (23). Groups were selected based on presence or absence of family history of atopy and the early milk-feeding pattern of each child. Positive family history of atopy was defined as asthma or atopic symptoms from two different organs in one or both parents. Atopic and nonatopic groups were further divided according to early feeding pattern either into a short (<0.5 mo) or long (>3.5 mo) exclusive breast-feeding group. The differences in duration of both exclusive and total breast-feeding were highly significant between the groups with short and long breast-feeding (Table 1, $p < 0.0001$).

The four study groups were invited to a visit at our outpatient clinic. Children in group A had a positive family history of atopy and long exclusive breast-feeding; in group B, negative family history of atopy and long exclusive breast-feeding; in group C, positive family history of atopy and short breast-feeding; and, in group D, negative family history of atopy and short breast-feeding (Table 1). The breast-feeding durations were quite similar in the groups with long (A and B) or short (C and D) breast-feeding (Table 1). The number of children taking part in the clinical study was 84 in group A, 63 in B, 73 in C, and 65 in D (5). We had a colostrum sample available from 64 (76%), 55 (87%), 58 (79%), and 51 (78%) mothers from groups A, B, C, and D, respectively.

Data collection. Data on atopic symptoms and the environment of each child came from the questionnaires filled in by parents when their children were born and at ages 2, 6, and 12 mo. At age 4 y, a new questionnaire was sent to the families to update the information on atopic symptoms. The investigator (M.S.) studied the children at the outpatient clinic and checked the questionnaire.

Definition of atopy. At age 4 y, skin-prick testing was performed with a panel of 11 standard Soluprick solutions from ALK (Allergologiska Laboratorium, Copenhagen, Denmark). A blood sample was taken for the measurement of serum total and allergen-specific IgE levels [birch, cat, CM, hen's egg, and house dust mite (*Dermatophagoides pteronyssinus*)], measured by enzymatic UniCAP fluoroimmunoassay. Serum total IgE levels >130 kU/L, and allergen-specific IgE levels >0.6 kU/L were considered positive. If any of the skin-prick test or serum IgE measurements was positive, the child was classified as IgE sensitized (5).

The child was considered to have atopic eczema if she or he had a history of chronic or chronically relapsing itching dermatitis with typical morphology and distribution. Diagnosis of allergic rhinitis or conjunctivitis was based on a history of runny or blocked nose or itchy, watery eyes or both with seasonal variation or with animal contact, and apart from infection episodes. Diagnosis of asthma required at least three episodes of bronchial obstruction reversed by a bronchodilatator and confirmation by a pediatrician. If the child had had any of the above-mentioned symptoms, she or he was considered to have symptoms of atopy. When a history of symptomatic atopy was validated by IgE sensitization, the child was classified as having verified atopy.

Colostrum collection. Samples of colostrum were collected from mothers on d 1–4 postpartum. Each sample was frozen within 12 h of collection and kept frozen at –70°C. After thawing, the sample was centrifuged at 10,000 *g* for 30 min, the cellular debris and fat layer were discarded, and the clear middle layer was used for analyses.

Measurements of total IgA, CM- and casein-specific IgA, TGF-β1 and -β2, and sCD14 in colostrum. Total concentration of IgA was measured by an immunoturbidimetric method using monospecific antisera to human IgA. IgA antibodies to whole CM in colostrum were measured by ELISA (24). Casein antibodies were measured with a similar method in microtitre wells coated with 2 μg/mL of commercial α-casein (Sigma Chemical Co., St. Louis, MO).

Concentrations of TGF-β1 and -β2 in breast colostrum were measured with the Quantikine Human TGF-β1 and TGF-β2 Immunoassays (R & D Systems, Minneapolis, MN). Activation of TGF-β1 in colostrum was performed as described for cell culture supernatants with 1N HCl (1/5 of the sample

Table 1. *Characteristics of study groups*

Group	Number	Duration of exclusive BF, months mean (range)	Duration of total BF, months mean (range)	Symptoms of atopy present N (%)	IgE sensitization positive N (%)	Verified atopy (Symptoms and IgE sensitizations) N (%)
Whole group	228	2.5 (0–6.5)	8.0 (0.5–36.0)	116 (50.9)	75 (32.9)	41 (18.0)
Long BF	119	4.4 (3.5–6.5)	10.3 (4.0–36.0)	59 (49.6)	40 (33.6)	21 (17.6)
Short BF	109	0.2 (0–0.5)	5.5 (0.5–30.0)	56 (51.4)	35 (32.1)	20 (18.3)
Positive FA	122	2.5 (0–6.0)	7.9 (0.5–30.0)	70 (57.4)	46 (37.7)	30 (24.6)
Negative FA	106	2.5 (0.1–6.5)	8.2 (0.8–36.0)	46 (43.4)	29 (27.4)	11 (10.4)
Long BF, positive FA	64	4.5 (4.0–6.0)	10.2 (5.0–30.0)	33 (51.6)	24 (37.5)	14 (21.9)
Long BF, negative FA	55	4.4 (3.5–6.5)	10.4 (4.0–36.0)	27 (49.1)	16 (29.1)	7 (12.7)
Short BF, positive FA	58	0.1 (0–0.5)	5.3 (0.5–5.3)	37 (63.8)	22 (37.9)	16 (27.6)
Short BF, negative FA	51	0.2 (0.1–0.5)	5.6 (0.8–22.0)	19 (37.3)	13 (25.5)	4 (7.8)

BF, breast feeding; FA, family atopy.

volume) for 10 min and neutralized with 1.2 N NaOH/0.5 M HEPES.

SCD14 levels were measured with an ELISA kit from IBL Inc. (Hamburg, Germany, catalog number RE 592 71) according to instructions of the manufacturer.

Data analysis. All measurements were transformed to logarithmic values to correct for the non-normal distribution. Geometric means and their 95% confidence intervals were calculated. In the whole study group as well as among those with long or short breast-feeding or positive or negative family history of atopy. Values of children with no atopic symptoms or IgE were compared with those of children with allergic symptoms and of children with symptoms and IgE (verified atopy).

The parameters measured for breast milk were dichotomized, and independent associations of these factors as well as that of length of breast-feeding and a family history on atopy were analyzed by multivariate stepwise logistic regression analysis by the forward selection method. Associations with symptoms of atopy and verified atopy were studied among the whole study group, those with long or short breast-feeding, and those with or without family history for atopy. Statistical analysis was done with the SPSS software package (version 11 for Windows, Chicago IL). Statistical significance was interpreted as two-sided alpha values <0.05.

Ethics. The study was approved by the Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki. Informed consent from the parents of the children to participate in the study was obtained.

RESULTS

Concentrations of total IgA, IgA CM, and casein antibodies, of TGF-β1 and -β2, and sCD14 in colostrum of atopic and nonatopic mothers. Mothers with any symptom suggestive of atopy had significantly lower concentrations of sCD14 in the colostrum than mothers without atopic symptoms. When mothers with asthma or two or more symptoms were compared with those nonatopic, the difference was no longer significant (Table 2).

Risks for verified atopy. In the whole study group, negative family history for atopy significantly reduced risk for verified atopy [odds ratio (OR), 0.32; 95% confidence interval (CI), 0.14–0.72; $p = 0.006$]. This was due to the reduction of risk among those with short breast-feeding (OR, 0.16; 95% CI, 0.04–0.67; $p = 0.012$), whereas, among those with long breast-feeding, the reduction was not significant (OR, 0.56; 95% CI, 0.2–2.0; $p = 0.45$). Low concentration of IgA casein antibodies in colostrum was a significant risk for verified atopy in the whole group (OR, 2.36; 95% CI, 1.05–5.3; $p = 0.04$). Among the children having had long breast-feeding, a low concentration of sCD14 tended to increase the risk for verified atopy (OR, 3.4; 95% CI, 1.0–12; $p = 0.06$).

Concentrations of total IgA, IgA CM, and casein antibodies, of sCD14, and of TGF-β1 and -β2 in colostrum. Figures 1–5 show geometric mean levels and 95% CI for total IgA, IgA CM antibodies, IgA casein antibodies, sCD14, and TGF-β2 in the whole study group, and among those with either short or long breast-feeding classified on the basis of atopy of children at age 4 y. Further, significant findings among those with or without a family history of atopy and in the single groups are presented. Comparison of concentration of total IgA to atopic findings of the child at age 4 y showed no difference in the whole study group (Fig. 1). IgA was significantly higher in colostrum of mothers with short breast-feeding whose children showed verified atopy ($p = 0.016$). In the group with long breast-feeding and negative family atopy, those mothers whose children showed verified atopy had a lower IgA concentration ($p = 0.033$).

In the whole study group and among the groups divided according to long and short breast-feeding, concentrations of IgA CM antibodies were similar in samples from mothers of atopic and nonatopic children (Fig. 2). In the colostrum of mothers whose children developed no atopy, IgA casein antibodies were significantly higher than in that of mothers with children with atopic symptoms ($p = 0.01$) or with verified atopy ($p = 0.009$) (Fig. 3). In long breast-feeding groups, IgA casein antibodies among mothers with children showing verified atopy were significantly lower than among those without atopy ($p = 0.023$, Fig. 3), whereas among those with short breast-feeding, differences were nonsignificant. Among the groups with a family history of atopy, samples from mothers whose children had either atopic symptoms or verified atopy showed lower levels of IgA casein antibodies than did those from mothers of children without atopy ($p = 0.029$ and 0.019; Fig. 3).

Children with verified atopy in the long breast-feeding groups had received breast milk with significantly lower concentration of sCD14 than did those without atopic symptoms ($p = 0.028$, Fig. 4). The same difference was significant among children in groups without family atopy ($p = 0.048$).

Table 2. *Concentrations of IgA cow's milk and casein antibodies, total IgA, TGF-β1 and -β2, and soluble CD14 in the colostrum of mothers classified by their symptoms of atopy*

	No atopic symptoms		At least one atopic symptom			Asthma or two or more atopic symptoms		
	N	Mean (95% CI)	N	Mean (95% CI)	p*	N	Mean (95% CI)	p*
IgA CM antibodies	119	3.3 (2.5–4.4)	109	2.7 (2.0–3.7)	0.30	83	2.4 (1.7–3.4)	0.17
IgA casein antibodies	119	1.1 (0.8–1.5)	109	1.2 (0.9–1.7)	0.60	83	1.3 (0.9–1.8)	0.53
Total IgA	118	2.6 (2.2–3.1)	109	2.7 (2.2–3.2)	0.85	83	2.8 (2.2–3.5)	0.61
TGF-β1	118	447 (376–531)	109	417 (347–500)	0.57	84	537 (363–525)	0.86
TGF-β2	94	3162 (2529–3954)	89	3090 (2574–3710)	0.10	73	3236 (2660–3936)	0.80
s-CD14	112	83.2 (77.9–88.8)	93	77.6 (73.6–81.9)	0.04	72	77.6 (73.6–81.8)	0.11

* Compared to the group without atopic symptoms by t-test.

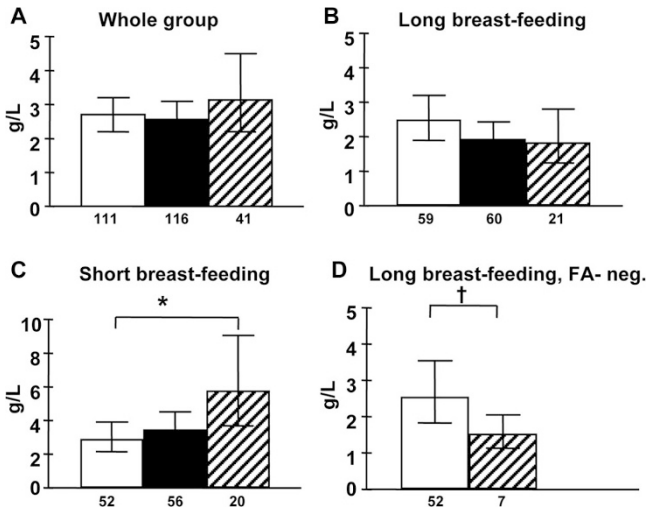

Figure 1. Geometric mean and 95% CI of concentration of total IgA in colostrum of mothers with children having no symptoms of atopy ([▨]), with one or more atopic symptoms (■), or with verified atopy (□, symptoms and IgE sensitization) in the whole group (*A*), among those with either long (*B*) or short (*C*) breast-feeding. These three groups are shown in all graphs (panels *A–C*). (*C*) Among those with short breast-feeding, a significant difference between those with verified atopy ($n = 20$) and those with no symptoms of atopy ($n = 52$). * $p = 0.016$. (*D*) In the group with long breast-feeding and negative family history of atopy (FA-neg.), a significant difference between those with verified atopy ($n = 7$) and no symptoms of atopy ($n = 52$). † $p = 0.033$.

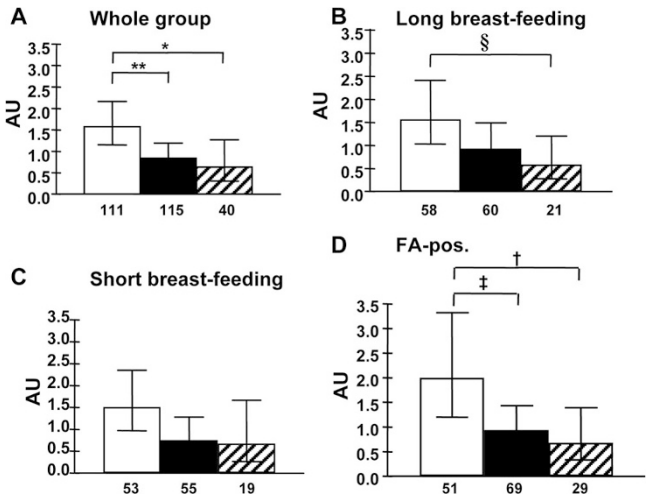

Figure 3. (*A–C*) Geometric mean and 95% CI of concentration of IgA casein antibodies (arbitrary units = AU) in the same groups as in Figure 1; atopy (▨), symptoms of atopy (■), verified atopy (□). (*A*) In the whole group, significant differences between those with verified atopy ($n = 40$) or those with symptoms of atopy ($n = 115$) and those with no symptoms of atopy ($n = 111$) (* $p = 0.009$ and ** $p = 0.01$, respectively). (*B*) Among those with long breast-feeding, a significant difference between those with verified atopy ($n = 21$) and those with no symptoms of atopy ($n = 58$) (§ $p = 0.023$). (*D*) Significant differences in the groups with positive family history of atopy (FA-pos.) between those with verified atopy ($n = 29$) or those with symptoms of atopy ($n = 69$) and those with no symptoms of atopy ($n = 51$) (†$p = 0.019$ and ‡ $p = 0.029$, respectively).

TGF-β1 concentrations in the colostrum samples from mothers with children with or without atopy did not differ (data not shown). Neither did TGF-β2 concentrations differ in the whole study group or among children classified according to length of breast-feeding (Fig. 5).

DISCUSSION

We had an opportunity to study soluble factors in colostrum samples of mothers of children studied for allergy and IgE sensitization at the age of 4 y. We show that defense factors in colostrum were relevant for the development of atopic symptoms and IgE sensitization at age 4 y. Among the factors, specific IgA antibodies to CM, measured against whole formula or casein, showed the closest association. Colostrum samples of mothers whose infants showed later atopy had a significantly lower level of IgA casein antibodies than did samples from mothers of nonatopic children, when infants had been breast-fed for a long period.

Samples from mothers of infants having symptoms suggestive of CMA were found to have lower levels of IgA CM antibodies than those from mothers whose infants had no symptoms (6). In two small series (25,26) and in our large series on 118 infants with CMA (13), however, milk of mothers whose infants developed CMA had levels of CM-specific IgA antibodies similar to those of mothers of infants tolerating CM. The present study indicates the importance of CM antigen–specific IgA antibodies in the colostrum in the development of later allergies and IgE sensitization, and further clarifies that the effect of breast milk–derived IgA is modified by the length of breast-feeding and family history of atopy; low concentrations of specific IgA were strongly associated with the risk of atopy when breast-feeding was long and infant had genetic predisposition to atopy.

In contrast to an earlier study reporting that nonallergic mothers' colostrum had higher levels of IgA antibodies against ovalbumin than did colostrum of allergic mothers, whereas levels against β-lactoglobulin and cat allergen were similar (7), we found no difference, either in total IgA concentration or in CM-specific IgA levels between allergic and nonallergic mothers. Instead, the levels of sCD14 were lower in colostrum

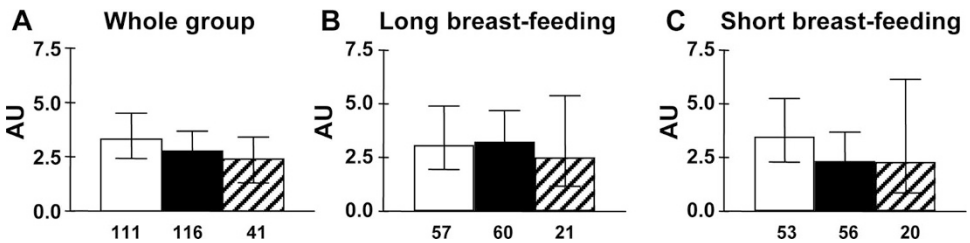

Figure 2. (*A–C*) Geometric mean and 95% CI of concentration of IgA antibodies to cow's milk (arbitrary units = AU) in the same groups as in Figure 1; atopy (▨), symptoms of atopy (■), verified atopy (□)

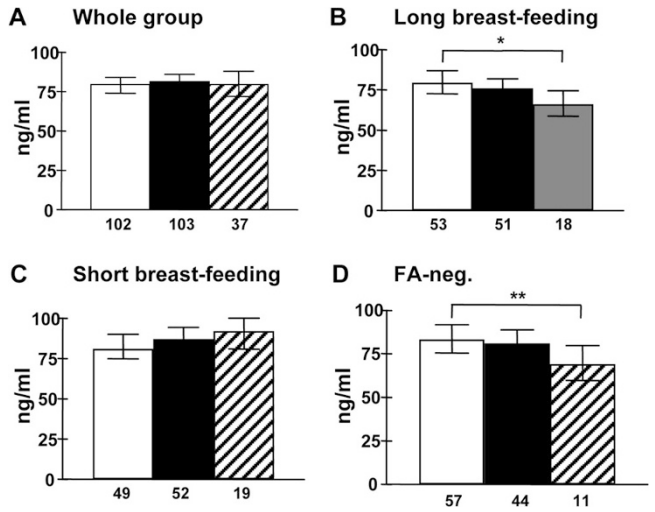

Figure 4. (*A–C*) Geometric mean and 95% CI of concentrations of soluble CD14 (ng/mL) in the same groups as in Figure 1; atopy (▨), symptoms of atopy (■), verified atopy (□). (*B*) Among those with long breast-feeding, a significant difference between those with verified atopy ($n = 18$) and those with no symptoms of atopy ($n = 53$). * $p = 0.028$. (*D*) A significant difference in groups with negative family history of atopy (FA-neg.) between those with verified atopy ($n = 11$) and those with no symptoms of atopy ($n = 57$) ** $p = 0.048$.

samples of atopic than nonatopic mothers when the definition of atopy was based on a single symptom. When a more rigid definition was used, the difference disappeared. Children having atopic symptoms and IgE sensitization at age 4 y had received colostrum with a significantly lower concentration of sCD14 than had those without symptoms and IgE sensitization, a difference present in groups with long breast-feeding and among those without family atopy, irrespective of length of breast-feeding. Jones and co-workers (27) found that infants with eczema had received at age 3 mo breast milk with lower sCD14 than did those without eczema and also showed lower amniotic fluid CD14 levels to be associated with later atopy. SCD14 is a co-receptor with a toll-like receptor 4 for lipopolysaccharide from Gram-negative bacteria needed for CD14-negative cells, such as intestinal epithelial and dendritic cells to respond to these products (28). The intensity of these responses during infancy may be important in strengthening the Th-1 responses and, in that way, counteracting the development of IgE-mediated allergic reactions. This relation between CD14 and IgE production was further suggested by the finding of a mutation in chromosome 5q31.1, which, when homozygous, resulted in higher levels of serum sCD14 and lower IgE levels (29). In addition, CD14 interacts directly with T and B cells, enhancing the secretion of IgG1 and reducing IgE secretion (30).

Breast milk contains a number of cytokines and other regulators of immune responses (11–17), and these, importantly, may influence the immune system of the newborn infant. In this study, we measured concentrations of TGF-β1 and -β2, cytokines with the highest concentrations in milk and the only ones shown to affect the newborn's immune function (13,31). We showed earlier that lower concentrations of TGF-β1 were associated with IgE-mediated CMA, and that the strength of immune responses to CM is associated with its concentration in colostrum (13). In another study, higher concentrations of TGF-β2 in colostrum were associated with IgA food antigen-specific B cells during infancy (31). In experimental animals, TGF-β in breast milk can rescue TGF-β knockout mice (32), and it plays an important role in the class switching to IgA production (33). A recent study showed an association between infantile wheezing and low concentrations of TGF-β1 in breast milk (22). In the present study, we found no significant associations between atopic symptoms or verified atopy at age 4 y and concentrations of TGF-β1 or -β2 in colostrum, suggesting that the effect of these factors does not extend beyond infancy.

In contrast to an earlier finding (21), we found no difference in the level of TGF-β2 between mothers with allergic diseases and those without. Another reported difference was the higher concentration of IL-4 in breast milk of allergic mother (15), a difference absent from the other study (34). Total IgA and CM-specific IgA in the milk of mothers with or without allergic diseases have been similar (26,35), and this was true also in the present study. Differences in the concentration of immunologically active substances in breast milk have been speculated to explain the greater impact of maternal than paternal allergy on the offspring (19), but the present findings do not support this hypothesis.

Two small studies have shown that colostral samples from mothers with infants developing CMA contained less IgA than did milk from mothers of nonallergic infants (25,26). In a large, population-based study, we found no difference in IgA concentration between milk from 110 mothers of CM allergic infants and 200 controls (13). Earlier studies showed that level of total IgA did not affect development of allergies up to the age of 5 y in 168 children (35) or among 53 children followed to the age of 2 y (36). The present study shows, among long breast-fed children with no heredity for atopy, an association with low total IgA in breast milk and verified atopy. However,

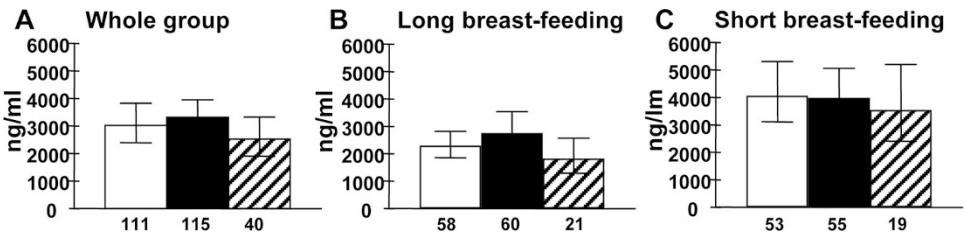

Figure 5. (*A–C*) Geometric mean and 95% CI of concentrations of TGF-β2 in the same groups as in Figure 1; atopy (▨), symptoms of atopy (■), verified atopy (□).

if breast-feeding duration was short, higher total IgA was found in breast milk of mother of infants with verified atopy. These infants were exposed exclusively to breast milk only <2 wk, and the effects of breast milk factor among such infants may be insignificant. This was the only significant association in the short breast-feeding group and in contrast to that seen in long breast-feeding group and may be a random finding.

CONCLUSIONS

We show that concentrations of factors present in maternal colostrum associate with atopic findings in children as late as at the age 4 y. Low levels of CM-specific IgA antibodies and sCD14 in colostrum imply a significant risk to develop atopic symptoms and IgE sensitization. This risk becomes apparent when an infant receives exclusive breast-feeding for >3.5 mo. Mothers with atopy showed no deficiencies of soluble factors in colostrum.

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