

Transforming growth factor- β in breast milk: A potential regulator of atopic disease at an early age

Marko Kalliomäki, MD,^a Arthur Ouwehand, PhD,^b Heikki Arvilommi, MD, PhD,^c
Pentti Kero, MD, PhD,^a and Erika Isolauri, MD, PhD^a *Turku, Finland*

Background: According to data from animal and in vitro studies, transforming growth factor- β (TGF- β) has a crucial effect on 2 essential parts of the mucosal immune system: IgA production and oral tolerance induction.

Objective: We sought to ascertain whether TGF- β in breast milk is associated with specific IgA production and atopic disease in human subjects.

Methods: Forty-seven infants with several atopic family members were followed during their first year of life. The concentrations of TGF- β 1 and TGF- β 2 in maternal colostrum, mature milk, and the infants' sera were determined. The enzyme-linked immunospot assay was used to assess the infants' specific IgA production in response to β -lactoglobulin, casein, gliadin, and ovalbumin.

Results: At 12 months, atopic dermatitis was confirmed in 29 of 47 infants; in 11, atopic disease had begun during exclusive breast-feeding (preweaning onset), whereas in 18 the disease manifested itself after weaning (postweaning onset). The concentrations of both TGF- β 1 and TGF- β 2 were higher in maternal colostrum, but not in mature milk and infants' serum, in infants with postweaning-onset atopic disease compared with those with preweaning-onset disease ($P = .0008$ and $P = .015$, respectively). The concentration of TGF- β 2 was, and that of TGF- β 1 tended to be, higher in the colostrum of mothers whose infants had specific IgA-secreting cells at 3 months in response to at least one of the dietary antigens tested compared with those who did not have such cells ($P = .048$ and $P = .076$, respectively).

Conclusion: TGF- β in colostrum may prevent the development of atopic disease during exclusive breast-feeding and promote specific IgA production in human subjects. (*J Allergy Clin Immunol* 1999;125:1-7.)

Key words: Transforming growth factor β , breast milk, atopic disease, immunoglobulin A, oral tolerance

The atopic diseases have been on the increase both in prevalence and severity during recent decades.¹ As to severity, atopic disease may manifest itself during the first months of life, even during exclusive breast-feeding, thus considerably predisposing to the subsequent development

Abbreviations used

ASC:	Antibody-secreting cell
DBPCCMC:	Double-blind, placebo-controlled cow's milk challenge
ELISPOT:	Enzyme-linked immunospot assay
IQR:	Interquartile range
ISC:	Immunoglobulin-secreting cell
TGF- β :	Transforming growth factor- β

of allergic airway disease² and interfering with normal growth and development.³ Early sensitization has been explained by the presence in breast milk of maternal dietary antigens in low amounts, ranging from picograms to nanograms per milliliter depending on the antigen measured and the method used.⁴⁻⁶ Defective low-dose oral tolerance induction to such antigen exposure may explain atopic disease during exclusive breast-feeding.⁷

Oral tolerance is a fundamental immunologic mechanism by which the immune system maintains unresponsiveness to nonpathogenic antigens previously encountered on mucosal surfaces (eg, from the gut lumen).⁸ Oral tolerance to low-dose antigen feeding is characterized by the induction of regulatory T cells producing suppressor cytokines, such as transforming growth factor- β (TGF- β). TGF- β is a multifunctional growth factor belonging to a superfamily of structurally related regulatory proteins. There are 5 different isoforms of this molecule, 3 of which (TGF- β 1, TGF- β 2, and TGF- β 3) are expressed in mammals.⁹ Human milk contains TGF- β 1 and TGF- β 2 at both messenger (m)RNA and protein levels, with TGF- β 2 being the major isoform.^{10,11} TGF- β has been shown both in vitro and in animal studies to have an important effect on IgA production.^{12,13} Hence TGF- β may be a key mediator in 2 essential parts of the mucosal immune system (ie, IgA production and oral tolerance induction). According to animal studies, the impact of TGF- β in breast milk may culminate at an early age, when new antigens are first encountered by the enteric route and the production of endogenous TGF- β in the gut is minimal.¹⁴

We hypothesized that TGF- β in maternal milk may be a decisive factor in diminishing the risk of early sensitization and atopic disease during breast-feeding. We followed 47 infants at high risk of atopic disease during their first year of life and determined the concentrations of TGF- β 1 and TGF- β 2 in maternal colostrum, mature milk, and the infants' sera. The enzyme-linked immunospot

From the Departments of ^aPaediatrics and ^bBiochemistry and Food Chemistry, University of Turku, and ^cthe National Public Health Institute, Turku. Supported by the Academy of Finland; the Foundation for Pediatric Research, Finland; and the Technology Development Centre of Finland.

Received for publication June 14, 1999; revised Aug 31, 1999; accepted for publication Sep 1, 1999.

Reprint requests: Marko Kalliomäki, MD, Department of Paediatrics, University of Turku, PO Box 52, FIN-20521 Turku, Finland.

Copyright © 1999 by Mosby, Inc.

0091-6749/99 \$8.00 + 0 1/1/102685

assay (ELISPOT) was used to assess the infants' specific IgA production in response to the dietary antigens β -lactoglobulin, casein, gliadin, and ovalbumin.

METHODS

Subjects and study design

The study involved a cohort of 47 voluntary families with 47 newborn infants who were randomly selected from the population of an allergy prevention study evaluating the preventive potential of probiotics in allergy. In this study population 19 (40%) of 47 infants had one and 28 (60%) of 47 had several first-degree family members with atopic eczema, allergic rhinitis, or asthma.

The study was approved by the Committees on Ethical Practice in Turku University Central Hospital and the Health Office of the City of Turku. Written informed consent was obtained from the children's parents.

The infants were born between the 36th and 43rd week of gestation (median, 40 weeks). They were clinically examined at the ages of 2 days, 2 weeks, and 3, 6, and 12 months. Any signs and symptoms of atopic disease were recorded. The diets of lactating mothers and infants were recorded as previously described.³ Atopic sensitization was evaluated by skin prick tests at the age of 12 months. Double-blind placebo-controlled cow's milk challenges (DBPC-CMC) were applied when symptoms, clinical signs, or skin prick test results were suggestive of cow's milk allergy.

Two venous blood samples were obtained by cubital venipuncture at the age of 3 months. The lithium-heparinized blood sample was analyzed by the ELISPOT method, and serum from the other sample was isolated for TGF- β analyses. A maternal colostrum sample was obtained immediately after commencement of nursing. A mature milk sample was obtained when the infant was 3 months old. Colostrum and milk samples were frozen and stored at -20°C until analyzed for the determination of TGF- β 1 and TGF- β 2 levels.

Diagnosis of atopic disease and cow's milk allergy

At the age of 12 months, the infants were grouped as having or not having atopic disease. The age of onset of atopic disease was determined at scheduled visits. Specifically, those with atopic disease were classified as having a preweaning-onset atopic disease if the disease had set in during exclusive breast-feeding or a post-weaning-onset atopic disease if disease manifested itself after weaning. Infants free of atopic disease were evaluated as a nonatopic control group.

The diagnosis of atopic dermatitis was based on criteria described elsewhere.¹⁵ Briefly, atopic dermatitis was confirmed if at least 3 of 4 major features were detected: pruritus, typical morphology and distribution, chronic or recurrent dermatitis, and a family history of atopy.

The diagnosis of cow's milk allergy was based on an unambiguous relationship between ingestion of cow's milk and clinical symptoms (ie, if symptoms disappeared after elimination of cow's milk from the diet and an unequivocal relapse occurred in a DBPCCMC). DBPCCMCs were carried out as described elsewhere.¹⁶

Evaluation of atopic sensitization

Atopic sensitization was determined by skin prick testing as previously described.¹⁶ Briefly, skin prick testing was carried out on the volar side of the forearm with a 1-mm, 1-peak lancet (Allergologisk Laboratorium A/S (ALK), Horsholm, Denmark) with a shoulder to prevent deeper penetration. Ten milligrams per milliliter histamine dihydrochloride (ALK) was used as positive control. Reactions were read at 15 minutes, and half of the histamine reac-

tion size (2+) or more was recorded as positive on the condition that the mean diameter of the wheal was at least 3 mm, and the negative control (ALK) at the same time was 0 mm. Antigens tested included a milk-containing 1.5% fat (Valio, Ltd, Turku, Finland), egg white (ALK), and wheat flour diluted 1:10 (wt/vol) with 0.9% (wt/vol) sodium chloride.

Determination of TGF- β levels in milk

We were able to get 43 maternal colostrum and 38 mature milk samples. Both samples were obtained from 35 mothers, and 1 sample only was obtained from 12 mothers. The milk and colostrum samples were thawed and subsequently centrifuged at 10,000g for 10 minutes to separate fat and cells from whey. The concentrations of total TGF- β 1 and TGF- β 2 in whey were determined by using commercial sandwich ELISAs specific to these molecules (R&D Systems Europe Ltd, Abingdon, UK). Latent TGF- β 1 was activated as described elsewhere.¹⁷ Briefly, the whey was incubated at room temperature for 1 hour with 1 mol/L HCl and neutralized with an equal volume of 1.2 mol/L NaOH/0.5 mol/L HEPES. Latent TGF- β 2 was activated as previously described.¹⁸ Briefly, the whey was incubated at room temperature for 1 hour with 1 mol/L acetic acid and neutralized with 2 volumes of 1.2 mol/L NaOH/0.5 mol/L HEPES. Other assays were performed as recommended by the manufacturer. Recovery in our spiking experiments was within the range reported by the manufacturer. All assays were performed in duplicate.

Determination of TGF- β levels in serum

We were able to obtain a serum sample for the TGF- β 1 analysis in 33 infants and TGF- β 2 analysis in 34 infants. The concentrations of total TGF- β 1 and TGF- β 2 in serum were determined by using commercial sandwich ELISAs specific to these molecules (R&D Systems Europe Ltd). Activation of latent TGF- β and all determinations were made according to the instructions provided by the manufacturer. All assays were performed in duplicate.

Enzyme-linked immunospot assay

The ELISPOT method was applied as described in greater detail elsewhere.¹⁹ The total number of immunoglobulin-secreting cells (ISCs) and that of antibody-secreting cells (ASCs) directed against dietary antigens were measured. In brief, mononuclear cells containing mainly lymphocytes (PBMCs) were obtained by Ficoll-Paque (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) centrifugation of lithium-heparinized blood. Isolated cells, which were washed 3 times in HBSS, were subsequently suspended in RPMI-1640 medium (Gibco Brl Life Technologies, Paisley, Scotland) containing 10% FCS (Gibco Brl, Life Technologies, Eggenstein, Germany) and adjusted to a concentration of 1 to 2×10^6 cells/mL.

To determine the number of specific ASCs against dietary antigens, coating was done for 2 hours at 37°C with the following concentrations of the antigens (all from Sigma Chemical Co, St Louis, Mo): 20 $\mu\text{g/mL}$ β -lactoglobulin from bovine milk, 20 $\mu\text{g/mL}$ casein from bovine milk, 20 $\mu\text{g/mL}$ gliadin, and 40 $\mu\text{g/mL}$ ovalbumin. Uncoated binding sites were blocked with 1% BSA (Boehringer Mannheim, GmbH, Germany) in PBS (pH 7.4) for 30 minutes at 37°C . After washing, the lymphocyte suspension was incubated on antigen-coated, flat-bottomed, 96-well microtiter plates (Immunoplate RI; A/S Nunc, Roskilde, Denmark) at 37°C for 2 hours. The antibodies secreted during that time were detected with alkaline phosphatase-conjugated goat anti-human IgA, IgG, and IgM antisera (all from Sigma Chemical Co) diluted in 1% BSA-PBS (pH 7.4), incubated overnight at room temperature, followed by a substrate agarose overlay and observation of the colored spots.

For the determination of ISCs, as an index of antigen-nonspecific immune response, the wells were coated with rabbit anti-human

TABLE I. Clinical characteristics of atopic children at the age of 12 months

	Preweaning onset* (n = 11)	Postweaning onset* (n = 18)	P value
Positive skin prick test responses			
For any antigen tested	6/11 (55%)	8/18 (44%)	.60†
Egg	6/11 (55%)	8/18 (44%)	.60†
Cow's milk	5/11 (45%)	2/18 (11%)	.07‡
Whey	2/11 (18%)	1/18 (6%)	.54‡
Positive DBPCCMC result	8/11 (73%)	10/18 (56%)	.45‡

*Atopic children were classified as having preweaning-onset atopic disease if atopic disease had begun during exclusive breast-feeding or postweaning-onset atopic disease if the onset was after weaning.

†Preweaning onset versus postweaning onset (χ^2 test).

‡Preweaning onset versus postweaning onset (Fisher's exact test).

IgA (Dako A/S, Glostrup, Denmark) and IgM (Dako A/S) and goat anti-human IgG (Sigma Chemical Co) diluted 1:100 in PBS (pH 7.4). Subsequent steps were the same as described above.

Statistical analysis

Because of skewed distribution, the data are expressed as medians with interquartile ranges (IQRs). The Fisher exact test or χ^2 test was applied to determine differences in contingency tables. The total number of patients with data in every table was over 20. The Fisher exact test was used if the total number of patients was between 20 and 40 and the smallest of the 4 expected values was less than 5. In other cases, the χ^2 test was applied. The Kruskal-Wallis test was used in comparisons between the groups; in case of statistical significance, the Mann-Whitney *U* test was used in pairwise comparisons. The Mann-Whitney *U* test and Wilcoxon signed-rank test were used in comparisons between 2 unpaired and paired groups, respectively. Correlation between 2 variables was calculated by using the Spearman rank correlation coefficient. A *P* value of less than .05 was considered statistically significant.

RESULTS

Clinical characteristics

At the age of 12 months, atopic dermatitis was confirmed in 29 (62%) of 47 children. None of the children had other manifestations of atopic disease. On the basis of our above-described criteria, 2 groups were identified: those with preweaning- and postweaning-onset atopic disease. In the former, atopic dermatitis had begun during exclusive breast-feeding at the median age of 2.5 months (IQR, 1.6-3.0 months) and in the latter after weaning at the median age of 4.3 months (IQR, 2.5-6.0 months; *P* = .0076).

The clinical characteristics of the atopic children are shown in Table I. Infants with preweaning-onset atopic dermatitis tended to have more frequently positive skin prick test reactivity to cow's milk than those with postweaning onset of disease, although the difference did not reach statistical significance. All the patients with cow's milk allergy had chronic symptoms of atopy. Isolated gastrointestinal reactions to cow's milk without skin symptoms were not seen. None of the control subjects had positive reactions to skin prick tests or cow's milk allergy. The number of infants with older siblings were 4 (22%) of 18, 4 (36%) of 11, and 5 (28%) of 18 infants who had no, preweaning, and postweaning onset of atopic disease, respectively (*P* = .71). The median (IQR) age of mothers whose infants had no,

preweaning, and postweaning onset of atopic disease was 30 years (27-34 years), 30 years (28-34 years), and 28 years (26-31 years), respectively (*P* = .15). None of the mothers were smokers. Furry pets were kept in 6 (33%) of 18, 2 (18%) of 11, and 2 (11%) of 18 homes of the respective infant groups (*P* = .25). Maternal atopic history was observed in 12 (67%) of 18, 7 (64%) of 11, and 16 (89%) of 18 infants with no, preweaning, and postweaning onset of atopic disease, respectively (*P* = .20).

Number of specific ASCs and nonspecific ISCs

We were able to obtain a blood sample for the ELISPOT at 3 months of age in 38 of 47 infants. The results of this assay are shown in Table II. Specific ASCs in response to at least one of the tested antigens (β -lactoglobulin, casein, gliadin, or ovalbumin) in the IgA, IgM, and IgG classes were observed in 31 of 38, 32 of 38, and 16 of 38 infants, respectively. Total numbers of ISCs were comparable in infants with and without specific ASCs to dietary antigens (Table II).

Concentrations of TGF- β in serum

The median (IQR) TGF- β 1 concentrations in serum were identical at the age of 3 months in control subjects and infants with preweaning- and postweaning-onset atopic disease: 61 ng/mL (45-80 ng/mL), 66 ng/mL (60-76 ng/mL), and 54 ng/mL (32-65 ng/mL), respectively (*P* = .28). The serum concentration of TGF- β 2 was lower than that of TGF- β 1, and again it did not differ between control subjects and infants with preweaning- and postweaning-onset atopic disease: 0.33 ng/mL (0.31-0.39 ng/mL), 0.34 ng/mL (0.28-0.42 ng/mL), and 0.32 ng/mL (0.31-0.34 ng/mL), respectively (*P* = .52).

Concentrations of TGF- β in breast milk

TGF- β 2 was the major form in colostrum and mature milk, representing up to 95% of TGF- β in these samples. The concentrations of TGF- β 1 and TGF- β 2 were significantly higher in colostrum compared with mature milk (Table III). The Spearman rank correlation between the concentrations in colostrum and mature milk of TGF- β 1 was 0.29 (*P* = .089), and that of TGF- β 2 was 0.10 (*P* = .55).

The concentrations of TGF- β 1 differed in the maternal colostrum of patients with no, preweaning, and post-

TABLE II. Infants with specific ASCs to dietary antigens and total numbers of ISCs in infants with and without specific ASCs to dietary antigens at the age of 3 months

	IgA	IgG	IgM
ASCs in response to*			
β-Lactoglobulin	21 (55%)	7 (18%)	21 (55%)
Casein	18 (47%)	6 (16%)	11 (29%)
Gliadin	12 (32%)	4 (11%)	24 (63%)
Ovalbumin	17 (45%)	7 (38%)	25 (66%)
Any antigens tested	31 (82%)	16 (42%)	32 (84%)
Total ISCs in infants			
With specific ASCs†	1020 (693-1448)	980 (550-1660)	375 (229-579)
Without specific ASCs‡	1760§ (1135-2058)	1195 (810-1410)	219¶ (140-720)

Numbers of ASCs and ISCs were assessed by using ELISPOT assay.

*Values represent the number (percentage) of the tested 38 infants having greater than 0 ASCs $\times 10^6$ PBMCs to a dietary antigen.

†Values represent median (IQR) numbers of ISCs (per 10^6 PBMCs) in 31, 16, and 32 infants with specific ASCs in IgA, IgG, and IgM classes, respectively.

‡Values represent median (IQR) numbers of ISCs (per 10^6 PBMCs) in 7, 22, and 6 infants without specific ASCs in IgA, IgG, and IgM classes, respectively.

§ $P = .13$, || $P = .52$, and ¶ $P = .47$ in statistical comparison between infants with specific ASCs versus infants without specific ASCs (Mann-Whitney U test).

TABLE III. Concentrations of TGF-β1 and TGF-β2 in colostrum and mature milk

	Colostrum	Mature milk	<i>P</i> value*
TGF-β1 (pg/mL)†	140 (67-186)	83 (17-114)	<.0001
TGF-β2 (pg/mL)†	3325 (1376-5394)	1644 (592-2697)	.0016

*Colostrum versus mature milk (Wilcoxon signed-rank test).

†Values represent median (IQR) concentrations in 43 colostrum and 38 mature milk samples.

weaning onset of atopic disease ($P = .0016$). This distinction was due to the higher concentrations of TGF-β1 in the colostrum of mothers of infants with postweaning onset of atopic disease compared with those with no and preweaning onset of disease ($P = .0056$ and $P = .0008$, respectively; Fig 1, A). The concentration of TGF-β2 in maternal colostrum also differed between these groups ($P = .04$), with the median concentration being significantly higher in the colostrum of mothers of infants with postweaning-onset disease compared with those with preweaning-onset disease ($P = .015$), while being comparable with that of the nonatopic control subjects (Fig 1, B). In mature milk these differences were no longer detectable (Fig 1, A and B).

As shown in Table IV, the median concentration of TGF-β2 was significantly higher in maternal colostrum in the case of infants who had specific IgA-secreting cells at the age of 3 months in response to at least one of the dietary antigens tested (β-lactoglobulin, casein, gliadin, or ovalbumin) compared with those who did not have such cells ($P = .048$). Again, the median concentration of TGF-β1 tended to be greater in the colostrum of mothers of infants with specific IgA-secreting cells than that in the colostrum of mothers of infants with no specific IgA-secreting cells ($P = .076$). The distinction was confined to the antigen-specific IgA response. The median (IQR) concentrations of TGF-β1 and TGF-β2 in the colostrum of mothers whose infants had ASCs in IgM class were 132 pg/mL (53-164 pg/mL) and 2370 pg/mL

(1250-4413 pg/mL), respectively. Compared with the concentrations in the colostrum of mothers whose infants did not have ASCs in IgM class (193 pg/mL [97-220 pg/mL] and 3224 pg/mL [1650-3738 pg/mL], respectively), there was no difference ($P = .17$ and $P = .73$, respectively). The median (IQR) concentrations of TGF-β1 and TGF-β2 in the colostrum of mothers whose infants had ASCs in IgG class were 145 pg/mL (60-171 pg/mL) and 2266 pg/mL (1067-4172 pg/mL), respectively. Again, compared with the concentrations in the colostrum of mothers whose infants did not have ASCs in IgG class (132 pg/mL [51-173 pg/mL] and 2830 pg/mL [1486-4413 pg/mL], respectively), no difference was detected ($P = .49$ and $P = .69$, respectively). Infants' exposure to these antigens through breast-feeding was considered similar, as determined by the mothers' dietary recordings. Moreover, none of the infants had been exposed to egg or cereals in their own diet.

DISCUSSION

TGF-β1 is considered indispensable for postnatal life.²⁰ The important postnatal immunoregulatory effect of milk TGF-β1 was substantiated in a recent study in which TGF-β1-null newborn mice were able to survive and develop normally only if TGF-β1 was present in maternal milk.²¹ Earlier studies have shown that breast milk contains TGF-β1 and TGF-β2 at both mRNA and protein levels.^{10,11} Our results would indicate that TGF-β in breast milk may also have a crucial role in the development of the mucosal immune system in the gut in human subjects.

The gut mucosal immune system is immature at birth. The intestinal mucosa is devoid of IgA, and the number of IgA-containing cells increases progressively until at least 4 years of age.^{22,23} TGF-β has a fundamental role in the production and class switching of IgA in vitro.¹² A recent study on an experimental animal model demonstrated a direct association between the expression of TGF-β in the lymphatic system and the mucosal IgA antibody response in vivo.¹³ In our study the concentra-

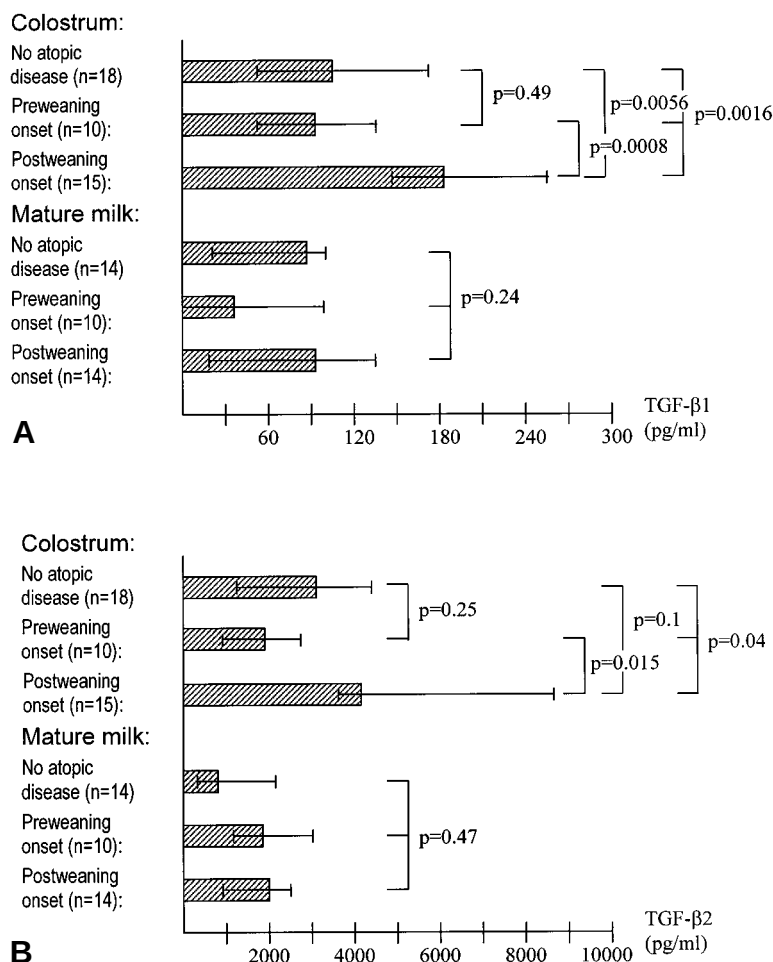


FIG 1. A, Concentrations of TGF-β1 in maternal colostrum and mature milk of infants with no, preweaning, and postweaning onset of atopic disease. *Columns* represent medians, and *errors bars* represent IQRs. **B,** Concentrations of TGF-β2 in maternal colostrum and mature milk of infants with no, preweaning, and postweaning onset of atopic disease. *Columns* represent medians, and *error bars* represent IQRs.

TABLE IV. Concentrations of TGF-β1 and TGF-β2 in maternal colostrum and mature milk in infants with or without specific IgA-secreting cells in response to dietary antigens

	Infants with specific IgA	Infants with no specific IgA	P value*
Colostrum†			
TGF-β1 (pg/mL)	145 (74-185)	53 (51-104)	.076
TGF-β2 (pg/mL)	3280 (1410-4792)	1489 (1123-1645)	.048
Mature milk‡			
TGF-β1 (pg/mL)	87 (15-121)	55 (21-94)	.48
TGF-β2 (pg/mL)	1840 (627-2779)	1215 (307-2087)	.70

*Infants with specific IgA versus infants with no specific IgA (Mann-Whitney *U* test).

†Values represent median (IQR) concentrations of TGF-β1 and TGF-β2 in maternal colostrum and mature milk of 28 infants with specific IgA-secreting cells and 7 infants with no specific IgA-secreting cells.

‡Values represent median (IQR) concentrations of TGF-β1 and TGF-β2 in maternal colostrum and mature milk of 24 infants with specific IgA-secreting cells and 6 infants with no specific IgA-secreting cells.

tion of TGF-β2 was significantly higher in the maternal colostrum in the case of infants with specific IgA-secreting cells in response to dietary antigens compared with those with no specific IgA-secreting cells. A similar trend was observed for TGF-β1. The difference was not

found in mature milk. Previous animal studies provide a potential explanation for this discrepancy. The endogenous TGF-β production increases with age, whereas the exogenous supply (ie, TGF-β in maternal milk) decreases with age.¹⁴ Moreover, TGF-β has been shown to have

an enhancing effect on its own production in vitro.²⁴ Thus TGF- β in early milk may potentiate its own production in the gut. As a consequence, endogenous TGF- β production in intestinal B cells and epithelial cells would then take over the regulation of mucosal IgA secretion.^{25,26} TGF- β in breast milk may be a starter of this process.

A key factor in oral tolerance induction appears to be the dose of antigen. Oral tolerance to high-dose antigen feeding is characterized by the induction of clonal deletion, anergy, or both, whereas that to low-dose antigen feeding is explained by the induction of regulatory T cells producing suppressor cytokines, such as TGF- β .^{8,27,28} It has been postulated that low-dose oral tolerance induction may occur in breast-fed infants receiving dietary peptides present in breast milk.⁷ Interestingly, we found lower concentrations of TGF- β 1 and TGF- β 2 in the colostrum of mothers of infants who already had atopic disease during exclusive breast-feeding compared with those who had atopic disease after weaning. Thus TGF- β in breast milk may act in preventing early atopic sensitization during exclusive breast-feeding by promoting low-dose oral tolerance induction as well as IgA generation in response to dietary antigens.

The ELISA kits we used have been thoroughly validated in studies of Ginjala and Pakkanen¹⁷ and Pakkanen¹⁸ in which bovine milk was used. In accord with data from earlier studies, we found the concentrations of both TGF- β 1 and TGF- β 2 to be significantly higher in colostrum compared with mature milk.^{10,11} The concentrations of TGF- β 1 and TGF- β 2 in our study were lower than those published by Saito et al,¹⁰ apparently because of the completely different methods used in the determination of TGF- β concentrations. In contrast, discrepancies in the concentrations of TGF- β in our study and that by Srivastava,¹⁷ in which no free TGF- β was detected, probably reflect differences in the preparation of breast milk samples (eg, centrifugation of samples before vs after freezing and differences in acid treatment of samples). We also demonstrated that the concentrations of TGF- β 1 and TGF- β 2 were comparable in the maternal colostrum in the case of infants in whom atopic disease developed during exclusive breast-feeding and that in nonatopic control subjects. It is thus clear that other mechanisms act, independently or in concert with TGF- β in breast milk, to prevent atopic disease.

Judging from our results, TGF- β in breast milk appears to be a potential regulator of early atopic sensitization. It may even be a decisive element in the successful primary prevention of allergy because of its potential effect on 2 essential parts of the mucosal immune system, namely IgA production and oral tolerance induction. A recent study showed TGF- β 1 and TGF- β 2 promoter polymorphisms in allergies and asthma.²⁹ In our study the number of mothers having personal atopic history was similar in infants who had no, preweaning, and postweaning onset of atopic disease. Our observation calls for identification of possible effects of specific atopic diseases on TGF- β in breast milk. More studies

are also needed to uncover the factors that determine TGF- β concentrations in breast milk and to establish whether these factors are amenable to therapeutic or preventive exploitation.

We thank Ms Pia Niemi and Ms Etta-Liisa Väänänen for technical assistance and Ms Johanna Hvitfelt-Koskelainen, RN, and Ms Ulla-Maija Eriksson, RN, for their contributions in the follow-up of study subjects.

REFERENCES

1. von Mutius E. The rising trends in asthma and allergic disease. *Clin Exp Allergy* 1998;28(Suppl 5):45-9.
2. Bergmann RL, Edenharter G, Bergmann KE, Forster J, Bauer CP, Wahn V, et al. Atopic dermatitis in early infancy predicts allergic airway disease at 5 years. *Clin Exp Allergy* 1998;28:965-70.
3. Isola E, Tahvanainen A, Peltola T, Arvola T. Breast-feeding of allergic infants. *J Pediatr* 1999;134:27-32.
4. Kilshaw PJ, Cant AJ. The passage of maternal dietary proteins into human breast milk. *Int Arch Allergy Appl Immunol* 1984;75:8-15.
5. Host A, Husby S, Hansen LG, Osterballe O. Bovine beta-lactoglobulin in human milk from atopic and non-atopic mothers. Relationship to maternal intake of homogenized and unhomogenized milk. *Clin Exp Allergy* 1990;20:383-7.
6. Sorva R, Mäkinen-Kiljunen S, Juntunen-Backman K. Beta-lactoglobulin secretion in human milk varies widely after cow's milk ingestion in mothers of infants with cow's milk allergy. *J Allergy Clin Immunol* 1994;93:787-92.
7. Pecquet S, Pfeifer A, Gauldie S, Fritsche R. Immunoglobulin E suppression and cytokine modulation in mice orally tolerized to betalactoglobulin. *Immunology* 1999;96:278-85.
8. Strober W, Kelsall B, Marth T. Oral tolerance. *J Clin Immunol* 1998;18:1-30.
9. McCartney-Francis NL, Frazier-Jessen M, Wahl SM. TGF-beta: a balancing act. *Int Rev Immunol* 1998;16:553-80.
10. Saito S, Yoshida M, Ichijo M, Ishizaka S, Tsujii T. Transforming growth factor-beta (TGF-beta) in human milk. *Clin Exp Immunol* 1993;94:220-4.
11. Srivastava MD, Srivastava A, Brouhard B, Saneto R, Groh-Wargo S, Kubit J. Cytokines in human milk. *Res Commun Mol Pathol Pharmacol* 1996;93:263-87.
12. Stavnezer J. Regulation of antibody production and class switching by TGF-beta. *J Immunol* 1995;155:1647-51.
13. Petitprez K, Khalife J, Cetre C, Fontaine J, Lafitte S, Capron A, et al. Cytokine mRNA expression in lymphoid organs associated with the expression of IgA response in the rat. *Scand J Immunol* 1999;49:14-20.
14. Penttälä IA, van Spriel AB, Zhang MF, Xian CJ, Steeb CB, Cummins AG, et al. Transforming growth factor-beta levels in maternal milk and expression in postnatal rat duodenum and ileum. *Pediatr Res* 1998;44:524-31.
15. Hanifin JM. Atopic dermatitis in infants and children. *Pediatr Clin North Am* 1991;38:763-89.
16. Isola E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J Allergy Clin Immunol* 1996;97:9-15.
17. Ginjala V, Pakkanen R. Determination of transforming growth factor-beta 1 (TGF-beta 1) and insulin-like growth factor (IGF-1) in bovine colostrum samples. *J Immunoassay* 1998;19:195-207.
18. Pakkanen R. Determination of transforming growth factor-beta 2 (TGF-beta 2) in bovine colostrum samples. *J Immunoassay* 1998;19:23-37.
19. Isola E, Virtanen E, Jalonen T, Arvilommi H. Local immune response measured in blood lymphocytes reflects the clinical reactivity of children with cow's milk allergy. *Pediatr Res* 1990;28:582-6.
20. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693-9.
21. Letterio JJ, Geiser AG, Kulkarni AB, Roche NS, Sporn MB, Roberts AB. Maternal rescue of transforming growth factor-beta 1 null mice. *Science* 1994;264:1936-8.
22. Perkkio M, Savilahti E. Time of appearance of immunoglobulin-contain-

- ing cells in the mucosa of the neonatal intestine. *Pediatr Res* 1980;14:953-5.
23. Haczek G, Savilahti E. Increase in density of jejunal IgA2 cells during infancy without change in IgA1 cells. *J Pediatr Gastroenterol Nutr* 1996;22:307-11.
24. Seder RA, Marth T, Sieve MC, Strober W, Letterio JJ, Roberts AB, et al. Factors involved in the differentiation of TGF-beta-producing cells from naive CD4+ T cells: IL-4 and IFN-gamma have opposing effects, while TGF-beta positively regulates its own production. *J Immunol* 1998;160:5719-28.
25. Christ AD, Blumberg RS. The intestinal epithelial cell: immunological aspects. *Springer Semin Immunopathol* 1997;18:449-61.
26. Zan H, Cerutti A, Dramitinos P, Schaffer A, Casali P. CD40 engagement triggers switching to IgA1 and IgA2 in human B cells through induction of endogenous TGF-beta: evidence for TGF-beta but not IL-10-dependent direct S mu—A alpha and sequential S mu—gamma, S gamma—alpha DNA recombination. *J Immunol* 1998;161:5217-25.
27. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994;265:1237-40.
28. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737-42.
29. Hobbs K, Negri J, Klennert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998;158:1958-62.

Receive tables of contents by e-mail

To receive the tables of contents by e-mail, send an e-mail message to
majordomo@mosby.com

Leave the subject line blank and type the following as the body of your message:
subscribe jaci_toc

You can also sign up through our website at
<http://www.mosby.com/jaci>

You will receive an e-mail to confirm that you have been added to the mailing list.
Note that TOC e-mails will be sent out when a new issue is posted to the website.