Acta Biochimica Polonica

Vol. 56 No. 3/2009, 411–414

on-line at: www.actabp.pl

Regular paper

TGF-β_1, IL-10 and IL-4 in colostrum of allergic and nonallergic mothers

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Received: 02 March, 2009; revised: 23 August, 2009; accepted: 09 September, 2009
available on-line: 14 September, 2009

Objective: To determine transforming growth factor (TGF) β_1, interleukin (IL) 4, and IL-10 concentrations in human milk and to assess the relationship between allergic disorders in mothers and the content of the interleukins in their milk. Material and methods: Thirty allergic and 46 healthy mothers were included in the study. Colostrum was collected 2–3 days after delivery. Cytokine concentrations were determined with commercial enzyme-linked immunosorbent systems. Results: TGF-$\beta_1$was found in milk from 23 women in the control group (53.49%) and 11 in the allergy group (37.93%). When TGF-β_1 was present, the median concentration was higher in the allergy group than in the control (61.5 and 30.4 pg/mL, respectively; $P<0.004$). IL-10 was present in the colostrum of all the women and the median IL-10 concentration did not differ between the allergy (50.5 pg/mL) and control (51.5 pg/mL) groups. The probability of occurrence of a positive IL-4 value in the allergy group was greater than in the control group (chi-squared [df=1] = 2.60, $P<0.053$). Median IL-4 level did not differ significantly between the two groups (0.5 and 0.5 pg/mL respectively). Conclusions: TGF-β_1 was detected less often in the colostrum of allergic mothers than in that of mothers without allergy (but the difference was not statistically significant). IL-4 was found more often in the colostrum of allergic mothers than nonallergic ones. The allergy status did not correlate with IL-10 concentration.

Keywords: allergy, human milk, IL-10, IL-4, TGF-β_1

INTRODUCTION

The immature immune system of neonates and infants leaves them susceptible to both infectious pathogens and environmental allergens. Breastfeeding, at least in the first six months of life, is believed to be of crucial importance for the normal functioning of an infant's organism. Human breast milk not only provides the infant with nutrients of adequate quality for growth and energy, but it also contains many cellular and humoral factors that form innate, antigen-nonspecific immunity (Newburg, 2005) and influence the development of mechanisms associated with acquired, antigen-specific immunity (Goldman, 1993; Kelly & Coutts, 2000; Hanson *et al.*, 2001; Field, 2005). Immunoactive components of breast milk may also influence late but long-lasting effects such as tolerance to nutritional antigens and physiological bacterial flora of the intestine. It can be assumed that disturbances in the development of oral tolerance underpin the pathophysiologic mechanisms related to the development of allergy and autoimmune diseases.

In spite of numerous studies of natural feeding, its precise role in preventing allergy in children remains unclear. Although breastfeeding is recom-

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Abbreviations: IL-4, interleukin 4; IL-10, interleukin 10; TGF-β_1, transforming growth factor β_1.

mended for children with a family history of allergy, especially if such history is on the mother's side, publications concerning the effects contain discrepant data and raise considerable debate (Kalliomaki *et al.,* 1999; Saarinen *et al.,* 2000; Hanson *et al.,* 2003; Friedman & Zeiger, 2005). In the light of the data collected to date, the question of whether or not the occurrence of allergy in the mother may affect the quantitative and qualitative composition of immunoactive factors in her milk seems of particular importance because it may reflect either a way of preventing or of promoting allergy development in children.

Additionally, among the numerous and diverse immunologically active milk components, it is difficult to indicate those of particular importance in the possible development of allergy. Human milk is known to contain cytokines that show anti- and proinflammatory effects (Meki *et al.,* 2003), mainly those that display immunomodulating activity. Their concentration in milk is usually inversely proportional to neonate maturity: colostrum contains more cytokines than mature milk (Hawkes *et al.,* 2002) and the concentration in milk is unrelated to the mother's serum concentration (Garofalo *et al.,* 1995). Of particular importance is the fact that cytokines present in human milk are not digested in the stomach but rather maintain their biological activity in the digestive tract of the child (Field, 2005). A potential relationship between the presence of certain cytokines in human milk and allergy occurrence in children who are breastfed has received considerable interest, but findings are inconsistent and subject to debate (Bottcher *et al.,* 2003; Rigotti *et al.,* 2006; Snijders *et al.,* 2006).

Taking into consideration the key roles of transforming growth factor (TGF)-β_1, interleukin (IL) 10, and IL-4 in the development of immune responses, we investigated the content of these cytokines in human milk. The aim of the study was to determine TGF-β_1, IL-4, and IL-10 concentrations in human breast milk and to assess the relationship between allergic disorders in women and the concentrations of these interleukins in their milk.

MATERIAL AND METHODS

Thirty allergic women in the puerperium period were included in the study. They were obstetrics department patients with previously diagnosed allergic disorders such as asthma (10 women), atopic dermatitis (7), or allergic rhinitis (13). The mean (S.D.) age in the allergic group was 27.6 years (5.12 years). The course of pregnancy and labor was normal in all the women. Except for allergic conditions, the patients had no other medical problems. The control group consisted of 46 healthy women who were also in the puerperium period and who had not had a complicated pregnancy or labor. The mean patient age in this group was 29.4 years (4.51 years). Colostrum was obtained for analysis 2 to 3 days after delivery. Immediately after sampling, the material was frozen at –20°C and stored at this temperature until analysis.

Patients gave written consent for participation in the study, which was approved by the independent bioethics committee of the Medical University of Gdańsk.

Colostrum cytokine concentrations (IL-4, IL-10 and TGF-β_1) were determined with use of a commercial enzyme-linked immunosorbent system (Quantikine Immunoassay, R&D System (MN, Minnesota, USA). The sensitivity thresholds of the kits were 0.11 pg/mL for IL-4, 3.09 pg/mL for IL-10, and 7 pg/mL for TGF-β_1.

Statistical analysis. The statistical analysis include comparing medians with the U Mann-Whitney test, testing hipotesis about the probability of success in independent trials, testing the independency of quality variables with Fisher exact test, Pearson chi-squared test and testing the Pearson correlation coefficients' (denoted by r) vanishing.

RESULTS

The concentrations of the cytokines analyzed in the colostrum are presented in Table 1. The differences in the percentage of TGF-β_1 positivity were not significant (for allergy and control 37.9% and 53.5%, respectively), although the median concentration of TGF-β_1 among allergic women with positive readings for this cytokine was significantly higher than the median concentration for the positive control group (Mann-Whitney test, $P<0.004$). IL-10 was present in colostrum in all women in both groups and the difference in the median concentrations of this cytokine between the groups was not significant. The probability of occurrence of a positive IL-4 value in the allergy group was greater than the frequency of a positive IL-4 value occurrence in the control group (chi-squared [df=1] = 2.60, $P<0.053$). Although IL-4 concentrations above the detection threshold (positive) were significantly more frequent in the allergy group than in the control group, the median concentrations did not differ significantly between the groups.

There was no significant correlation between positive results for TGF-β_1 and IL-4 in the control group (60.87% women with positive TGF-β_1 were IL-4 positive and 55.0% women with negative TGF-β_1 were IL-4 positive). In contrast, allergic women in whose colostrum TGF-β_1 was positive all had posi-

Table 1. Concentrations of TGF-β_1, IL-4 and IL-10 in colostrum.

Results of analyses	TGF-β_1		IL-4		IL-10	
	Control (n = 46)	Allergy (n = 30)	Control (n = 46)	Allergy (n = 30)	Control (n = 46)	Allergy (n = 30)
Negative, %[a]	46.51	62.07	41.3	23.3	0	0
Positive, %[a]	53.49	37.93	58.7	76.7[b]	100	100
Median (S.D.) concentration, pg/mL	30.4 (20.5)	61.5[c] (74.8)	0.36 (0.37)	0.33 (0.34)	51.5 (80.4)	50.5 (97.5)

Abbreviations: IL, interleukin; TGF, transforming growth factor. [a]A positive result was the concentration of a cytokine above the detection limit; a negative result was a concentration below the detection limit; [b]The probability of occurrence of a positive IL-4 value in allergy group is greater than in the control group (chi-squared [df=1] = 2.60, $P<0.053$). [c]The difference between median concentration of TGF-β_1 among allergic women and control group $P<0.004$ (Mann-Whitney U test).

tive IL-4 and only 61.11% of women with negative TGF-β_1 had positive IL-4. Thus the presence of Il-4 was correlated with the presence of TGF-β_1 (Fisher exact test, $P<0.026$).

A Pearson correlation between TGF-β_1 and IL-4 concentrations (when they were positive) was noted in the control group (the higher the concentration of TGF-β_1, the higher the concentration of IL-4, r=0.45, $P<0.025$) whereas in the allergy group an inverse relationship was observed (the higher the concentration of TGF-β_1, the lower the concentration of IL-4, r=–0.32, $P<0.141$). The relationship between TGF-β_1 and IL-4 in the allergy group was not statistically significant, in contrast to the relation we observed in the control group.

When comparing colostrum concentrations of TGF-β_1 and IL-10 we did not observe Pearson correlation coefficients statistically significantly different from 0.

Considering the relation between IL-4 and IL-10, a statistically significant relationship was observed in the control group (r=0.44, $P<0.021$) whereas in the allergy group (r=0.34) it was not statistically significantly different from 0.

DISCUSSION

Papers published in recent years present discrepant results on possible relationships between the occurrence of atopic conditions in women and TGF-β_1, IL-10, and IL-4 content in their milk. Conclusions also vary concerning the effect of the presence and concentrations of these cytokines in human milk on the development of allergy in breastfed children. Thus, since the first publication on the presence of TGF-β_1 in human milk (Zwiebel *et al.*, 1986) this issue is still being investigated, yet results remain discrepant and do not clearly explain the role played by this cytokine in allergy prevention in children.

In the colostrum samples that we examined, TGF-β_1 tended to be present more frequently in the control group than in atopic women but the difference was not significant. However, if women with negative results were excluded from the analysis, it was found that TGF-β_1 concentrations in the colostrum reached higher values in women with allergy, and the difference in comparison with such women in the control group was significant. These results are different from those reported by Laiho *et al*. (2003) and Bottcher *et al.* (2003) who found this cytokine in the colostrum of all examined mothers, with lower concentrations in allergic women than in the control group. Rigotti *et al.* (2006), in turn, concluded that TGF-β_1 concentrations in the colostrum of allergic women do not differ significantly from concentrations in the colostrum of women without allergy, but in the mature milk of allergic women these concentrations were significantly lower.

Although the mean IL-10 concentrations in the combined group of all women in our study were higher than the mean results obtained by Meki *et al*. (2003), our results are consistent with theirs in that we did not find any statistically significant differences between the mean IL-10 concentrations of women with and without allergy.

In contrast to our results, Rigotti *et al*. (2006) found IL-10 in the colostrum of only a few women with allergy, and in amounts much smaller than those revealed by our research. Also Bottcher *et al*. (2003) found IL-10 in only 12% of the colostrum samples examined. According to those authors, the results might be attributable to delayed (3–4 days after labor) test material collection. These differences seem to be the result of different sensitivities of the test methods used. The detection threshold for our IL-10 determination method was much lower than that of Bottcher and coworkers. Snijders *et al*. (2006), who examined human milk one month after labor, did not find detectable amounts of IL-10 at all with assays with a detection threshold of 0.2 pg/mL.

When examining the IL-4 content of allergic women's colostrum, we obtained positive results significantly more frequently than for the control group although, the median concentrations in the two groups were not significantly different. The study of Bottcher *et al*. (2000) showed that although colostrum of allergic women contains significantly higher

concentrations of IL-4 than the colostrum of women without allergy, this difference becomes statistically non-significant for mature milk. Laiho *et al*. (2003), in similar groups of women, did not find IL-4 in their milk at all. However, those authors performed their study as late as the second and third month of lactation.

It is undoubtedly possible that the results of study could have been influenced by a number of factors such as a potential effect of the circadian rhythm on cytokine production or changes in the water content in milk. These issues have not been addressed in the literature thus far, however, and require further investigation. The discrepancies between our results and the earlier ones are mainly due to differences with respect to patients` selection criteria, different laboratory methods used, and to low subject numbers in the examined groups to date. Most authors point to a need for continued research of these issues. It seems, however, that only well-coordinated multicenter studies following a uniform design would be purposeful. Clarification of the actual significance of potential neonate and infant immune system immaturity compensation by immunologically active breast milk components may be a good reason for continuation of this line of research. We must not forget that among lymphocytes located in the lamina propria of the intestinal mucosa there are only few T_H3 and T_Hr lymphocytes that secret TGF-β_1 and IL-10 that are so important for the maintenance of a child's immune homeostasis (Beyer *et al*., 2002).

In summary, we found that TGF-β_1 was detected less often in the colostrum of allergic mothers than in that of mothers without allergy (but the difference was not statistically significant). IL-4 was found more often in the colostrum of allergic mothers than nonallergic ones. The allergy status did not correlate with IL-10 concentration.

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