

Perinatal Period Cytokines Related to Increased Risk of Future Allergy Development*

J. ŽIŽKA^a, M. KVERKA^b, O. NOVOTNÁ^a, I. STAŇKOVÁ^a, R. LODINOVÁ-ŽÁDNÍKOVÁ^c, I. KOCOURKOVÁ^c,
I. ŠTERZL^a, L. PROKEŠOVÁ^{a**}

^a*Institute of Immunology and Microbiology, 1st Faculty of Medicine, Charles University in Prague, 128 00 Prague, Czechia*

^b*Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czechia*

^c*Pediatric Department, Institute for the Care of Mother and Child, 147 00 Prague, Czechia*

fax +420 224 968 455

e-mail ludmila.prokesova@lf1.cuni.cz

Received 30 January 2007

Revised version 28 June 2007

ABSTRACT. Testing of cytokine levels in colostrum, cord blood and amniotic fluid of healthy and allergic mothers and their newborns (using protein microarrays and quantitative analysis by ELISA) revealed differences in the levels of IL-5, IL-10, TGF- β , TNF- α , EGF and eotaxin between healthy and allergic groups. Significantly higher concentration of IL-5 and IL-10 in the colostrum of allergic mothers and cord blood of their children and also tendency to a higher level of IL-4 found at allergic mothers and their children (but without statistical significance) indicate a bias to T_H2 response in this group. The higher level of TGF- β in the colostrum of healthy mothers should be involved in beneficial immunological tuning of their children including enhanced IgA formation and better intestine maturation. In amniotic fluid, concentration of TGF- β was higher in children of allergic mothers. A significantly higher level of EGF was proved in the colostrum of healthy mothers and in cord blood of their children in comparison with allergic group. EGF deficiency in the allergic group could impair or delay intestine maturation and support thus allergy development.

Abbreviations

EGF	epidermal growth factor	PDGF-BB	platelet-derived growth factor BB
ELISA	enzyme-linked immunosorbent assay	RANTES	regulated upon activation, normal T-cell expressed and secreted
GIT	gastrointestinal tract	SEM	standard error of the mean
GRO- α	growth related oncoprotein α	TGF- β	transforming growth factor β
IL	interleukin	T _H	helper T-lymphocyte
IFN- γ	interferon γ	TNF- α	tumor necrosis factor α
MCP-1	monocyte chemotactic protein-1	VEGF	vascular endothelial growth factor
MIP-1 δ	macrophage inflammatory protein 1 δ		

Apart from genetic predisposition in children of allergic parents, the development of allergy is promoted by a number of external factors acting on the developing immune system of the individual. Maternal milk, which is the main source of newborn's nutrition after birth, contains not only all necessary nutrients supporting growth, but also a broad range of immunologically active substances (interleukins, chemokines, interferons, antibodies, growth factors, hormones, *etc.*) and cells (epithelial cells, macrophages, neutrophils and lymphocytes) that can affect the processes occurring in the immune system of the newborn (Field 2005; Kverka *et al.* 2007; Newburg *et al.* 2007). Breast feeding of an infant by healthy mother is generally taken to be beneficial. Certain differences have been described between the composition of milk of healthy and allergic mothers; a question therefore arises if the milk of an allergic mother has equally beneficial effects as that of a healthy one (Duchén *et al.* 1999, 2000; Friedman *et al.* 2005).

Since various proteinaceous substances have been shown to be able to penetrate through the intestinal mucosal wall into the organism and a similar penetration of cells has been demonstrated on animal models (Liebler-Tenorio *et al.* 2002; Schnorr *et al.* 1984) and indirectly also in humans (Schlesinger *et al.* 1977), the still immature digestive tract of the newborn, with its increased permeability and lower ability to degrade food components can be assumed to allow the transfer of immunological components of mother's milk into the newborn's organism in their immunologically active form. They can then influence the immune system

*1st part of this study: *Pediatr. Allergy Immunol.* **17**, 175–183 (2006).

**Corresponding author.

of the child not only in intestinal mucosa but in the whole organism (Hanson *et al.* 2003; Kelleher *et al.* 2001).

However, even during the prenatal period the child is exposed to the action of various substances arriving from the mother and present in the amniotic fluid. The fetus swallows the amniotic fluid, whose components thus come into contact with the immature mucosae of the digestive and respiratory tracts. These substances can then be assumed to penetrate into the whole organism of the fetus and could influence the development and tuning of the immature immune system already in the prenatal period (Warner *et al.* 2000; Warner 2004).

To assess the possible differences in the influence of a healthy and allergic mother on the child's development, we compared the levels of some cytokines in colostrum and in amniotic fluid collected in mid-gestation during diagnostic amniocentesis performed in healthy and allergic mothers. We paid attention mainly to cytokines representative for T_H1 (IFN- γ) and T_H2 (IL-4–IL-6, IL-13) responses, cytokines with immunosuppressive function (IL-10, TGF- β), cytokines important for isotype switch (IL-4, IL-5, IL-10, TGF- β) and factors influencing maturation of intestinal wall (TGF- β , EGF).

The character of the cells and soluble components of newborn's blood is determined by both the genetic traits of the parents and the phenotypic properties of the mother's organism with which the fetus is in close contact throughout the gestation *via* the placenta and amniotic fluid. The newborn's blood would then allow us to assess the risk of future appearance of allergy. We therefore also tested a set of immunological markers in the serum from cord blood collected after the delivery. In an attempt to evaluate the prognostic significance of cord blood examination, we compared the cord blood composition of newborns from healthy mothers with that of newborns of allergic mothers, who pose a higher risk of future allergy.

MATERIALS AND METHODS

Subjects. Two groups of mothers and children according to mothers' clinical statement were used as donors:

- (1) healthy individuals – non-allergic mothers and newborn children of non-allergic mothers;
- (2) allergic individuals – allergic mothers and newborns of allergic mothers.

The study was approved by the *Ethical Committee of the Institute for the Care of Mother and Child* and was carried out with the informed consent of the mothers. Diagnostics of allergy in mothers was based on clinical manifestation of allergy persisting for >2 years (allergy against respiratory and food allergens manifested by various individual combinations of hay fever, conjunctivitis, bronchitis, asthma, eczema, *etc.*), monitoring by an allergologist, positive skin prick tests or positive specific IgE and anti-allergic treatment before pregnancy.

Tested samples. Colostrum specimens were collected on the 3rd day after delivery. Mothers expressed their breast milk manually into sterile polypropylene tubes and stored it for 1–2 h at 4 °C until delivered to laboratory; the fat layer and cell pellet were then removed by centrifugation (8000 g, 25 min, 4 °C) and soluble fraction was stored in aliquots at –20 °C until processed.

Cord blood samples were collected in sterile tubes immediately after delivery with the usual procedures including careful cleaning of the cord and puncture of umbilical vein to avoid maternal contamination. Serum obtained after centrifugation (1500 g, 10 min, 4 °C) was stored at –20 °C.

Amniotic fluid samples were obtained by amniocentesis made for diagnostic purposes in the 20th week (± 1 week) of pregnancy. All samples were centrifuged (8000 g, 10 min, 4 °C) to eliminate insoluble parts and kept frozen at –20 °C.

Cytokine estimation by protein array method. RayBio Human Cytokine Array III (Raybiotech, USA) was used for simultaneous detection of a large number of cytokines in one sample (Table I). Array membranes were processed according to producer's recommendation. LAS-1000 detector (Fujifilm, Japan) was used for detection of luminescence and the data were then analyzed using AIDA 3.28 software (Raytest, Germany). After subtracting the background staining, relative protein levels normalized to positive controls (applied on the membrane by the manufacturer) were obtained for each single membrane.

Cytokine estimation by ELISA method. Cytokines (IL-4–IL-6, IL-8, IL-10, IL-12, IL-13, IFN- γ , TGF- $\beta 1$, eotaxin, MCP-1, GRO- α , RANTES, TNF- α and EGF) were quantified by ELISA using high-adSORption polystyrene microtitration plates (Nunc, Denmark). Paired antibodies, where the second antibody was biotinylated (R&D System, Germany), were used according to producer's recommendation. The results were read from calibration curves of recombinant cytokines (R&D System) using Genesis software and expressed in pg/mL.

Table 1. Map of the RayBio Human Cytokine Array II membrane used for detection of cytokines in individual samples of amniotic fluid, colostrum and cord blood serum^a

No.	A	B	C	D	E	F	G	H	I	J	K	L
1	PC	PC	NC	NC	ENA-78	G-CSF	GM-CSF	GRO	GRO- α	I-309	IL-1 α	IL-1 β
2	PC	PC	NC	NC	ENA-78	G-CSF	GM-CSF	GRO	GRO- α	I-309	IL-1 α	IL-1 β
3	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12p40p70	IL-13	IL-15	IFN- γ
4	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12p40p70	IL-13	IL-15	IFN- γ
5	MCP-1	MCP-2	MCP-3	M-CSF	MDC	MIG	MIP-1 β	RANTES	SCF	SDI-1	TARC	TGF- β 1
6	MCP-1	MCP-2	MCP-3	M-CSF	MDC	MIG	MIP-1 β	RANTES	SCF	SDI-1	TARC	TGF- β 1
7	TNF- α	TNF- β	EGF	IGF-1	Ang	OSM	TPO	VEGF	PDGF BB	leptin	NC	NC
8	TNF- α	TNF- β	EGF	IGF-1	Ang	OSM	TPO	VEGF	PDGF BB	leptin	NC	NC

^aAng

angiogenin

OSM

oncostatin M

TPO

thrombopoietin

PC

positive control

NC

negative control

Statistics. The data are presented as means $\pm$ SEM for protein arrays or as medians and the 1st and the 3rd quartile for quantitative estimation by ELISA. Differences between groups were evaluated using unpaired Mann-Whitney test. Statistical significance was set at $p \leq 0.05$.

RESULTS

Cytokines present in amniotic fluid, cord blood and maternal colostrum were followed by protein microarrays and ELISA and their concentration in healthy mothers and their children was compared with that of allergic mothers and their children.

Relative quantification of large sets of cytokines performed by protein microarray did not prove any significant differences between the healthy and the allergic group. The summary of relative concentrations of the cytokines whose amount exceeded at least 10 % of positive controls in cord blood, amniotic fluid or colostrum is shown in Table II.

The results of analysis of selected cytokines performed by ELISA are summarized in Table III. Cytokines present in high quantities (medians ≥ 100 pg/mL) are listed *below*;

amniotic fluid: IL-6, IL-8, TGF- β , MCP-1, GRO- α ;

maternal colostrum: IL-4, IL-5, IL-10, IL-13, IFN- γ , TGF- β , TNF- α , MCP-1, GRO- α , EGF; concentration of EGF exceeded 10^5 pg/mL;

cord blood: IL-4, IL-5, IL-10, IL-12, IL-13, TGF- β , eotaxin, GRO- α , EGF, RANTES; concentrations of IL-13, TGF- β , EGF, eotaxin and RANTES exceeded 1000 pg/mL.

On comparing the healthy and allergic groups, the differences in IL-5, IL-10, TGF- β , TNF- α , EGF and eotaxin were found to be significant. Although no significant differences were found with IL-4, the allergic group showed the tendency to higher levels of this cytokine. The amount of IL-5 was significantly higher in the colostrum of allergic mothers ($p = 0.008$) and in the cord blood of their children ($p = 0.04$). Similarly, the colostrum of allergic mothers as well as the cord blood of their children contained significantly higher levels of IL-10 (in colostrum $p = 0.004$, in cord blood $p = 0.03$). The amount of TGF- β in the colostrum of allergic mothers was markedly lower than in that of healthy mothers ($p = 0.0009$); children of allergic mothers also displayed lower TGF- β level in the cord blood, though the difference was not significant due to large individual variability. By contrast, the concentration of TGF- β in amniotic fluid of children of allergic mothers was significantly higher than in the group of children of healthy mothers ($p = 0.02$). TGF- β was the only cytokine in amniotic fluid, in which ELISA documented marked quantitative differences between the healthy and the allergic group. TNF- α in the amniotic fluid was difficult to evaluate since its low level was at the limit of detection.

The amount of eotaxin in the cord blood of children of healthy mothers was much higher than in the offspring of allergic mothers ($p = 0.006$). A similar trend was found in the colostrum but the values were very low and were thus at the limit of detection. Contrary to the negligible level of EGF in amniotic fluid, a high amount of this growth factor was found in colostrum. The level of EGF in the colostrum of healthy mothers was signifi-

cantly higher than in allergic ones ($p = 0.04$). Higher amount of EGF ($p = 0.03$) was also in cord blood of children of healthy mothers (Table IV).

Table II. Relative levels of selected cytokines (exceeding at least 10 % of positive controls) in amniotic fluid (at the 20th week of gestation), colostrum (3rd day after delivery) and cord blood collected from individuals of healthy and allergic group detected by protein microarray method^{a,b}

Cytokine	Amniotic fluid		Cord blood		Colostrum	
	HI	AI	HI	AI	HI	AI
EGF	5.3 ± 2.0	5.6 ± 1.4	121 ± 35.2	105 ± 41.0	460 ± 137	432 ± 147
ENA-78	2.3 ± 1.0	2.7 ± 1.3	3.8 ± 1.8	4.7 ± 1.6	24.0 ± 12.1	34.9 ± 24.1
GRO	10.6 ± 7.7	8.9 ± 4.4	46.9 ± 21.0	7.9 ± 8.3	203.0 ± 177	183 ± 147
IL-6	52.4 ± 61.7	69.4 ± 77.7	0.9 ± 0.4	0.7 ± 0.9	5.1 ± 6.1	2.4 ± 1.6
IL-8	53.8 ± 49.2	36.6 ± 16.3	8.3 ± 3.4	10.1 ± 5.0	82.5 ± 46.9	102 ± 42.0
IGF-I	1.3 ± 0.6	1.7 ± 0.4	8.7 ± 6.3	6.6 ± 4.9	29.9 ± 24.7	25.9 ± 15.8
Leptin	42.3 ± 43.4	26.9 ± 22.6	22.6 ± 16.9	9.7 ± 9.2	2.6 ± 1.4	6.2 ± 4.6
MCP-1	13.6 ± 8.2	21.6 ± 8.0	8.7 ± 3.2	4.2 ± 2.3	159 ± 82.1	105 ± 43.9
MIP-1 δ	10.3 ± 5.8	10.8 ± 5.2	31.7 ± 19.3	20.5 ± 14.1	8.7 ± 6.3	10.2 ± 5.4
PDGF-BB	1.5 ± 0.5	1.9 ± 0.5	86.0 ± 37.2	63.7 ± 36.2	4.0 ± 0.7	3.3 ± 0.3
RANTES	5.0 ± 3.3	5.1 ± 3.4	49.5 ± 14.0	37.3 ± 7.4	4.4 ± 1.8	6.0 ± 1.1
VEGF	3.8 ± 1.2	4.3 ± 1.7	6.4 ± 2.4	5.0 ± 3.6	27.5 ± 16.0	25.5 ± 8.4
Angiogenine	60.5 ± 28.8	66.9 ± 14.9	139 ± 28.4	81.7 ± 30.9	207 ± 63.1	179 ± 41.4

^aMean values normalized to positive controls (*i.e.* 100 %) ± SEM of 8 samples (amniotic fluid) or 4 samples (cord blood, colostrum).

^bHI healthy individuals AI allergic individuals

DISCUSSION

The children of allergic parents (mainly of allergic mothers) are in a larger risk of future development of allergy (Peden 2005; Tariq *et al.* 1998). There is a great effort to define some prognostic markers to be able to anticipate the future ailment and to make possible preventive measures. Some differences in immunological indices in newborn children (mainly in cytokine concentrations) were reported but there are many controversies in this respect (Amoudruz *et al.* 2005; Hauer *et al.* 2003; Kopp *et al.* 2001). Many data concerning differences in milk composition of healthy and allergic mothers were published but they are also not unambiguous (Bottcher *et al.* 2000a,b; Snijders *et al.* 2006). The most reliable factor increasing the probability of the rise of allergy in a child has so far been the allergy of the mother. The decisive role of genetic background is generally accepted but it is well known that also environmental factors and phenotypic features of the mother influence the development of child's immune system and can modulate its tuning in the direction of allergy development (Peden 2005). The main influence of maternal organism is mediated by breast feeding. Amniotic fluid, the constituents of which are both of fetal and maternal origin, can exert a similar effect during intrauterine development of the fetus.

We intended to contribute to better characterization of factors signaling the increased probability of future allergy occurrence. For this reason we analyzed the cytokine spectrum in cord blood and maternal colostrum, and we also examined the amniotic fluid taken by amniocentesis in the middle of gestation for other diagnostic reasons which has as yet not been much addressed in connection with allergy.

The protein microarray method used for the first screening revealed relative levels of cytokines (normalized to positive controls present on each microarray membrane) in all three body fluids tested. This quantification can serve only for rough orientation since the detection sensitivity of individual cytokines is different. Also the comparison of healthy and allergic groups is unreliable because of the small number of samples we were able to test by this method. More exact comparison was done after quantitative estimation of selected cytokines using ELISA method.

Already at an early stage of intrauterine development, amniotic fluid is very rich in various lymphokines, chemokines and growth factors (Nowicki *et al.* 2005). The levels of T_H2 cytokines IL-4 and IL-6 are higher in amniotic fluids from the allergic group but the differences between allergic and healthy groups are not significant. The only significant difference between amniotic fluids of children of healthy and allergic mothers is the higher concentration of TGF- β in allergic group, which is a situation quite opposite to that in colostrum. TGF- β role in IgA formation, peripheral tolerance induction and intestine maturation implies the

importance of this cytokine for immunological tuning of the organism. However, its concentration both in amniotic fluid and colostrum is relatively low in comparison with the concentration in newborn's blood and its active synthesis is supposed to take place in newborn's organism (Prokešová *et al.* 2006); hence, TGF- β

Table III. Levels of cytokines in amniotic fluid (at the 20th week of gestation), in colostrum (3rd day after delivery), and in cord blood of children of healthy and allergic mothers detected by ELISA (pg/mL)^a

Cytokine	Healthy individuals				Allergic individuals				<i>p</i> ^b
	<i>n</i>	Q1	median	Q3	<i>n</i>	Q1	median	Q3	
Amniotic fluid									
IL-4	16	0	0	5.0	16	0	0	0.1	0.9231
IL-5	16	0.6	9.2	56.2	16	0.8	5.2	61.5	0.7914
IL-6	16	87.1	137	309	16	72.1	137	273	0.8065
IL-8	16	93.4	198	434	16	121	232	378	0.8459
IL-10	16	10.1	12.1	24.5	16	8.1	10.6	17.5	0.1343
IL-12	16	9.5	12.9	41.5	16	10.4	15.5	48.5	0.9335
IL-13	16	14.9	41.3	420	16	5.5	12.6	125	0.4282
IFN- γ	16	0	0	0.1	16	0	0.1	41.7	0.2209
TGF- β	16	45.0	262	382	16	325	602	1320	0.0195
Eotaxin	16	7.4	32.5	57.9	16	5.5	11.1	57.3	0.8830
MCP-1	16	284	432	1240	16	438	616	733	0.8911
GRO- α	16	231	305	604	16	184	326	602	0.5167
RANTES	16	4.3	10.2	32.8	16	1.6	4.0	8.5	0.0705
TNF- α	16	16.7	25.8	32.9	16	40.9	47.4	58.8	0.0013
EGF	16	1.1	1.8	4.5	16	1.5	2.0	7.9	0.3656
Colostrum									
IL-4	43	73.0	119	231	64	86	147	428	0.0851
IL-5	43	5.0	73.0	128	64	59.5	122	188	0.0079
IL-6	43	29.0	74.0	159	64	38.0	84.5	177	0.7315
IL-8	13	24.5	55.5	97.1	15	22.3	53.3	95.9	0.5140
IL-10	37	20.0	154	321	60	151	300	464	0.0042
IL-12	14	1.3	4.8	10.2	17	3.4	6.8	15.7	0.5589
IL-13	39	9.0	243	1900	63	39.0	397	1710	0.5375
IFN- γ	42	15.5	115	1120	63	47.0	211	577	0.7536
TGF- β	44	299	581	824	62	95.2	229	510	0.0009
Eotaxin	14	1.6	37.3	71.3	16	2.1	8.8	20.3	0.0203
MCP-1	16	142	225	528	24	106	217	468	0.5194
GRO- α	20	208	287	702	21	215	287	554	0.8081
RANTES	16	33.1	49.3	61.2	16	17.5	44.6	61.9	0.5820
TNF- α	15	11.0	258	301	15	12.0	163	271	0.7208
EGF	22	119×10^3	235×10^3	451×10^3	22	106×10^3	166×10^3	255×10^3	0.0431
Cord blood									
IL-4	42	21.5	132	276	55	91.0	167	386	0.0629
IL-5	42	31.5	86.5	127	55	71.0	108	198	0.0405
IL-6	41	41.0	63.0	127	55	25.3	45.6	120	0.7727
IL-8	43	40.7	58.2	80.4	43	43.2	65.8	120	0.3206
IL-10	36	25.5	141	315	50	135	238	369	0.0290
IL-12	14	140	151	180	13	131	153	165	0.6066
IL-13	38	89.5	732	2215	54	145	1400	3275	0.2046
IFN- γ	41	19.5	92.0	1455	55	44.6	69.3	300	0.4146
TGF- β	43	28×10^3	69×10^3	105×10^3	53	23×10^3	42×10^3	88×10^3	0.1250
Eotaxin	13	865	1275	1765	13	15.5	138	998	0.0060
MCP-1	14	2.0	8.0	12.5	13	1.0	5.0	124	0.9419
GRO- α	14	153	600	1010	13	66.5	250	672	0.0746
RANTES	14	9200	12×10^3	20×10^3	13	9845	16×10^3	21×10^3	0.5816
TNF- α	40	27.8	73.6	356	44	4.2	66.8	446	0.7505
EGF	40	743	1500	2540	44	701	1305	1945	0.0312

^a*n* – number of tested samples, Q1 – 1st quartile, Q3 – 3rd quartile.

^bStatistically significant results are in **boldface**.

received by swallowing amniotic fluid or colostrum cannot play any decisive role in immunological regulations in the newborn.

Table IV. Levels of EGF in amniotic fluid (at the 20th week of gestation), colostrum (at the 3rd day after delivery) and cord blood of healthy and allergic mothers detected by ELISA (pg/mL)^a

Sample	Healthy individuals				Allergic individuals				<i>p</i>
	<i>n</i>	Q1	median	Q3	<i>n</i>	Q1	median	Q3	
Amniotic fluid	16	1.1	1.8	4.5	16	1.5	2.0	7.9	0.3656
Colostrum	22	119 × 10 ³	235 × 10 ³	451 × 10 ³	22	106 × 10 ³	166 × 10 ³	255 × 10 ³	0.0431
Cord blood	40	743	1500	2540	44	701	1300	1940	0.0312

^aSee footnotes of Table III.

Significantly higher levels of IL-5 and IL-10 and the tendency to higher levels of IL-4 in colostrum of allergic mothers in comparison with healthy mothers point to an increased bias to T_H2 response in this group. The same trend is evident in sera of newborns of allergic mothers where IL-5 and IL-10 levels are significantly and IL-4 nonsignificantly higher. The increased level of IL-10 in allergic group was surprising. We suggest it could represent compensatory mechanism in the group with increased risk but still without allergy manifestation. We detected relatively low levels of IL-8 and RANTES in colostrum of both healthy and allergic mothers, which is not in correspondence with the data of Bottcher *et al.* (2000a) and Meki *et al.* (2003) who found high levels of IL-8 and RANTES in allergic mothers. These authors suggested that the high levels of IL-8 and RANTES in milk compensated delayed chemokine formation in newborns; however, we proved high RANTES levels in cord blood sera. The only difference between healthy and allergic mothers in cytokine composition in colostrum shown by Laiho *et al.* (2003) was a lower concentration of TGF-β2 in allergic mothers. We demonstrated a lower concentration of TGF-β1 as well but the role of colostral TGF-β is probably not decisive, as mentioned *above*.

The mediator which has not been studied in connection with allergy development is EGF. This growth factor is (beside other functions) extremely important for the development of the gastrointestinal tract (Cummins *et al.* 2002; Takeda *et al.* 2004); in amniotic fluid of mid gestation it is hardly detectable. Its concentration in cord blood is high but it is lower in children of allergic mothers than the level in newborns of healthy mothers (*p* = 0.03). Remarkably, EGF concentration in maternal colostrum is two orders of magnitude higher than the concentration in cord blood; this is in accordance with the positive role which is played by colostrum in GIT development. The lower EGF concentration in allergic colostrum (*p* = 0.04) could contribute to the possible delayed development of intestine of children breast-fed by allergic mothers; this could be a cause of increased intestinal permeability facilitating allergy induction. The results allow us to suggest the low levels of neonatal and colostral EGF as a factor pointing to increased probability of future development of allergy.

The low degree of significance in some cases where the trend is quite evident is caused by extensive individual variability.

This work was supported by the grant no. IGA/MZ NR/8040-3 of the *Ministry of Public Health of the Czech Republic*.

REFERENCES

- AMOUDRUZ P., HOLMLUND U., MALMSTROM V., TROLLMO C., BREMME K., SCHEYNIUS A., SVERREMARK-EKSTROM E.: Neonatal immune responses to microbial stimuli: is there an influence of maternal allergy? *J.Allergy Clin.Immunol.* **115**, 1304–1310 (2005).
- BOTTCHER M.F., JENMALM M.C., BJORKSTEN B., GAROFALO R.P.: Chemoattractant factors in breast milk from allergic and nonallergic mothers. *Pediatr.Res.* **47**, 592–597 (2000a).
- BOTTCHER M.F., JENMALM M.C., GAROFALO R.P., BJORKSTEN B.: Cytokines in breast milk from allergic and nonallergic mothers. *Pediatr.Res.* **47**, 157–162 (2000b).
- CUMMINS A.G., THOMPSON F.M.: Effect of breast milk and weaning on epithelial growth of the small intestine in humans. *Gut* **51**, 748–754 (2002).
- DUCHEN K., THORELL L.: Nucleotide and polyamine levels in colostrum and mature milk in relation to maternal atopy and atopic development in the children. *Acta Paediatr.* **88**, 1338–1343 (1999).
- DUCHEN K., CASAS R., FAGERAS-BOTTCHER M., YU G., BJORKSTEN B.: Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. *Pediatr.Allergy Immunol.* **11**, 29–39 (2000).
- FIELD C.J.: The immunological components of human milk and their effect on immune development in infants. *J.Nutr.* **135**, 1–4 (2005).

- FRIEDMAN N.J., ZEIGER R.S.: The role of breast-feeding in the development of allergies and asthma. *J.Allergy Clin.Immunol.* **115**, 1238–1248 (2005).
- HANSON L.A., KOROTKOVA M., LUNDIN S., HAVERSEN L., SILFVERDAL S.A., MATTSBY-BALTZER I., STRANDVIK B., TELEMÖ E.: The transfer of immunity from mother to child. *Ann.N.Y.Acad.Sci.* **987**, 199–206 (2003).
- HAUER A.C., RIEDERER M., GRIESSL A., ROSEGGER H., MACDONALD T.T.: Cytokine production by cord blood mononuclear cells stimulated with cows milk proteins *in vitro*: interleukin-4 and transforming growth factor β -secreting cells detected in the CD45RO T cell population in children of atopic mothers. *Clin.Exp.Allergy* **33**, 615–623 (2003).
- KELLEHER S.L., LONNERDAL B.: Immunological activities associated with milk. *Adv.Nutr.Res.* **10**, 39–65 (2001).
- KOPP M.V., ZEHLE C., PICHLER J., SZEPPALUSI Z., MOSELER M., DEICHMANN K., FORSTER J., KUEHR J.: Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production. *Clin.Exp.Allergy* **31**, 1536–1543 (2001).
- KVERKA M., BURIANOVÁ J., LODINOVÁ-ŽÁDNÍKOVÁ R., KOCOURKOVÁ I., ČÍNOVÁ J., TUČKOVÁ L., TLASKALOVÁ-HOGENOVÁ H.: Cytokine profiling in human colostrum and milk by protein array. *Clin.Chem.* **53**, 955–962 (2007).
- LAIHO K., LAMPI A.M., HAMALAINEN M., MOILANEN E., PIIRONEN V., ARVOLA T., SYRJANEN S., ISOLAURI E.: Breast milk fatty acids, eicosanoids, and cytokines in mothers with and without allergic disease. *Pediatr.Res.* **53**, 642–647 (2003).
- LIEBLER-TENORIO E.M., RIEDEL-CASPARI G., POHLENZ J.F.: Uptake of colostrum leukocytes in the intestinal tract of newborn calves. *Vet.Immunol.Immunopathol.* **85**, 33–40 (2002).
- MEKI A.R.M.A., SALEEM T.H., AL-GHAZALI M.H., SAYED A.A.: Interleukins-6, -8 and -10 and tumor necrosis factor- α and its soluble receptor I in human milk at different periods of lactation. *Nutr.Res.* **23**, 845–855 (2003).
- NEWBURG D.S., WALKER W.A.: Protection of the neonate by the innate immune system of developing gut and of human milk. *Pediatr.Res.* **61**, 2–8 (2007).
- NOWICKI S., GOLDBLUM R.M.: Amniotic fluid and the fetal mucosal immune system, pp. 1747–1762 in P.L. Ogra, J. Mestecky, M.E. Lamm, W. Strober, J. Bienenstock, J.R. McGhee, L. Mayer (Eds): *Mucosal Immunology, Vol. I*, 3rd ed. Elsevier Academic Press, San Diego 2005.
- PEDEN D.B.: The epidemiology and genetics of asthma risk associated with air pollution. *J.Allergy Clin.Immunol.* **115**, 213–219 (2005).
- PROKEŠOVÁ L., LODINOVÁ-ŽÁDNÍKOVÁ R., ŽIŽKA J., KOCOURKOVÁ I., NOVOTNÁ O., PETRÁSKOVÁ P., ŠTERZL I.: Cytokine levels in healthy and allergic mothers and their children during the first year of life. *Pediatr.Allergy Immunol.* **17**, 175–183 (2006).
- SCHLESINGER J.J., COVELLI H.D.: Evidence for transmission of lymphocyte responses to tuberculin by breast-feeding. *Lancet* **2**, 529–532 (1977).
- SCHNORR K.L., PEARSON L.D.: Intestinal absorption of maternal leucocytes by newborn lambs. *J.Reprod.Immunol.* **6**, 329–337 (1984).
- SNIJEDERS B.E., DAMOISEAUX J.G., PENDERS J., KUMMELING I., STELMA F.F., VAN R.R., VAN DEN BRANDT P.A., THIJIS C.: Cytokines and soluble CD14 in breast milk in relation with atopic manifestations in mother and infant (Koala Study). *Clin.Exp.Allergy* **36**, 1609–1615 (2006).
- TAKEDA T., SAKATA M., MINEKAWA R., YAMAMOTO T., HAYASHI M., TASAKA K., MURATA Y.: Human milk induces fetal small intestinal cell proliferation – involvement of a different tyrosine kinase signaling pathway from epidermal growth factor receptor. *J.Endocrinol.* **181**, 449–457 (2004).
- TARIQ S.M., MATTHEWS S.M., HAKIM E.A., STEVENS M., ARSHAD S.H., HIDE D.W.: The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study. *J.Allergy Clin.Immunol.* **101**, 587–593 (1998).
- WARNER J.A., WARNER J.O.: Early life events in allergic sensitisation. *Brit.Med.Bull.* **56**, 883–893 (2000).
- WARNER J.O.: The early life origins of asthma and related allergic disorders. *Arch.Dis.Child* **89**, 97–102 (2004).