

Cytokine levels in healthy and allergic mothers and their children during the first year of life

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To assess the regulatory changes of immune system in children genetically pre-disposed to allergic diseases and in their mothers, we tested cytokines IL-4, IL-5, IL-6, IL-10, IL-13, IFN- γ and TGF- β in 21 healthy and 21 allergic mothers (serum at the time of delivery, colostrum and milk throughout the suckling period) and their children (cord blood, venous blood and stool filtrates) up to 1 yr of age. Samples were taken at the time of delivery, 4 days post-partum and then after 3, 6 and 12 months. Significant differences between the healthy and the allergic group were found in the levels of IL-4, IL-10, IL-13 and IFN- γ . The levels of IL-4 in the allergic group were generally higher; the levels in the sera of children of allergic mothers during the post-natal life decreased, reaching levels typical for the healthy group at 1 yr of age. Allergic mothers exhibited markedly higher IL-10 levels in the serum at the time of delivery and in milk 3 months after delivery than healthy mothers while after 6 months the IL-10 levels in all samples from the allergic group were very low. Children from allergic group had lower intestinal content of IL-13 in comparison with the healthy counterparts. At 1 yr of age, the levels of IFN- γ in sera and stool of children from the allergic group sharply increased. TGF- β levels in the sera of both groups were high, while in the milk they were relatively low and substantially lower than in the children's stool. TGF- β of mammary secretions is therefore unlikely to exert a decisive regulatory influence on the children's immunity. Long-term clinical monitoring of the children will be performed to evaluate the potential prognostic significance of these changes for the future development of allergies.

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Allergic diseases, which are on the rise, represent a considerable medical, social and economic problem. The genetic pre-disposition to these ailments is clear (1–3). However, the child not only receives genetic information from its parents, but the development of its immune system is affected during the whole pre-natal and early post-natal life by the immune system of the mother and later by environmental factors. Maternal colostrum milk contain a number of humoral and cellular components that affect the regulations of the child's

immune responses (4, 5). The beneficial effect of breast-feeding on the protection of the child against infections is clearly documented and well known (6, 7). It is also generally assumed that breast-feeding reduces the occurrence of future allergic diseases in children (8). This is indeed the case when the child is breast-fed by a healthy mother. However, it is still a matter of conjecture if the breast-feeding by allergic mother is equally beneficial (8, 9). To answer this question and to formulate possible recommendations on the length of breast-feeding, it is

necessary to know in more detail the immunological functions of maternal milk and possible differences between the milk of healthy and allergic mothers. This study presents the results of testing of cytokine levels in healthy and allergic mothers and their children; it includes tests performed with serum, colostrum and milk of the mothers and cord blood, venous blood and stool filtrates of their children. The first tests concerned cytokines that have a direct relationship to the development of the atopic response (IL-4, IL-6 and IL-13), production of IgA (IL-5, IL-6, IL-10 and TGF- β) and some cytokines with known regulatory

function (IFN- γ , IL-4, IL-10 and TGF- β) (10–12). The children were simultaneously followed clinically in order to reveal the potential onset of an allergic condition. The whole study is conceived as a long-term one, with the present communication summarizing results corresponding to the first year of the children's life.

Methods

The study was performed on a group of healthy mothers and their children ($n = 21$) and allergic mothers and their children ($n = 21$). Children of the latter group were genetically pre-disposed to allergies because their parents were allergic – mothers in all cases, fathers and grandparents in some cases. The study was approved by the Ethical Committee of the Ministry of Public Health and was carried out with the informed consent of the mothers.

Allergy diagnostics in mothers

(a) allergic clinical manifestations persisting for more than 24 months, (b) monitoring by an allergologist (collaboration with allergologist,

Table 1. Numbers of breastfed and non-breastfed children in individual time intervals

Time after delivery	Healthy mothers and their children		Allergic mothers and their children	
	Breastfed	Non-breastfed	Breastfed	Non-breastfed
4 days	21	0	21	0
3 months	19	2	16	5
6 months	18	3	13	8
12 months	12	9	6	15

Table 2. IL-4 levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	21	190	39–405	21	292	181–1,313	0.0593
	4 days	19	105	91–197	21	372	213–1,454	0.0007
	3 months	21	52	0–139	20	306	164–833	0.0003
	6 months	21	73	0–152	21	190	126–271	0.0066
	12 months	20	112	19–165	17	48	24–208	0.5523
Children's stool filtrates	4 days	21	125	28–386	21	279	153–840	0.0111
	3 months	21	73	6–211	20	323	117–1,133	0.0034
	6 months	21	39	3–209	21	173	82–502	0.0429
	12 months	20	130	88–264	19	50	18–347	0.4651
Mother's serum	Time of delivery	21	184	37–284	20	607	338–1,562	0.0006
Mother's milk	4 days	21	172	53–261	20	235	134–1,250	0.1177
	3 months	18	83	13–180	14	362	92–1,031	0.0088
	6 months	16	61	25–114	11	248	84–363	0.0318
	12 months	12	120	66–234	4	82	10–228	0.6713

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	19	105			21	372		
	3 months	19	52	2	55	16	228	4	788
	6 months	18	69	3	87	13	190	8	252
	12 months	12	121	8	110	4	150	13	48
Children's stool filtrates	4 days	21	125			21	279		
	3 months	19	71	2	177	16	244	4	706
	6 months	18	46	3	34	13	153	8	173
	12 months	12	123	8	151	4	120	15	50

Table 3. IL-5 levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	21	88	17–132	21	113	74–209	0.1189
	4 days	19	99	26–287	21	100	54–175	0.7349
	3 months	21	65	25–209	20	112	67–276	0.5573
	6 months	21	115	26–245	21	104	11–292	0.7917
	12 months	20	135	99–212	16	125	17–269	0.4641
Children's stool filtrates	4 days	21	124	32–188	21	124	49–285	0.5131
	3 months	21	79	20–317	20	88	19–131	0.9688
	6 months	21	183	83–417	20	268	93–593	0.6764
	12 months	20	220	119–861	19	253	83–5,327	0.8994
Mother's serum	Time of delivery	21	123	31–198	20	134	38–359	0.6294
Mother's milk	4 days	21	79	4–119	20	126	60–180	0.0622
	3 months	18	70	14–151	14	76	20–125	0.9243
	6 months	16	142	39–205	11	78	5–167	0.1324
	12 months	12	189	97–243	4	76	21–182	0.3631

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	19	99			21	100		
	3 months	19	57	2	647	16	97	4	193
	6 months	18	114	3	170	13	92	8	216
	12 months	12	159	8	107	4	94	12	131
Children's stool filtrates	4 days	21	124			21	124		
	3 months	19	43	2	482	16	88	4	242
	6 months	18	187	3	183	12	188	8	521
	12 months	12	191	8	378	4	319	15	226

written report), (c) positive reaction to antiallergic treatment, (d) positive skin tests or positive specific IgE. All mothers of allergic group had allergy against respiratory allergens (pollen, dust and mite) manifested with various individual combinations of hay fever, conjunctivitis, bronchitis, breathlessness, asthma, eczema and edema. All of them were under anti-allergic drug treatment prior to pregnancy and, in severe cases of allergy, also during nursing.

Samples tested

(a) Sera from maternal blood at the time of delivery, cord blood and children's venous blood, (b) Colostrum and milk after centrifugation (8,000 *g*) and fat removal. The numbers of samples are different in various time periods because breastfeeding was of different length in individual children under study. All children were breastfed at the beginning of the study. Table 1 gives the numbers of breastfed and non-breastfed children in individual periods, (c) Children's stool filtrates –1 g of stool was suspended in 1 ml PBS and centrifuged at

8,000 *g*, supernatants were used for analyses. Samples were taken at the time of delivery, 4 days after delivery and after 3, 6 and 12 months. All samples were stored at –20° C until use.

Cytokine estimation

Cytokines (IL-4, IL-5, IL-6, IL-10, IL-13, IFN- γ and TGF- β) were quantified by ELISA method on high-adsorption polystyrene microtitration plates (NUNC). Paired antibodies, where second antibody was biotinylated (R&D System, Wiesbaden, Germany), were used according to producer's recommendation. The results were read from calibration curves of recombinant cytokines (R&D System) using GENESIS software.

Result expression and statistics

Results are expressed as medians and percentiles (25%–75%). Significance was estimated using the Mann–Whitney test. $P \leq 0.05$ was considered significant.

Table 4. IL-6 levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	21	59	0–127	21	58	15–131	0.6148
	4 days	21	75	2–194	21	24	9–129	0.4205
	3 months	21	92	47–135	20	25	9–140	0.2106
	6 months	21	121	57–193	21	81	34–164	0.3520
	12 months	20	104	23–206	18	164	49–359	0.2141
Children's stool filtrates	4 days	21	75	11–142	21	42	9–131	0.5543
	3 months	21	91	53–144	20	35	11–117	0.0876
	6 months	21	110	62–204	21	91	38–184	0.4355
	12 months	20	89	29–162	19	180	47–223	0.1365
Mother's serum	Time of delivery	21	71	6–160	20	47	2–128	0.5157
Mothers milk	4 days	21	51	0–171	20	73	21–136	0.6072
	3 months	18	78	14–112	14	37	11–120	0.4233
	6 months	16	105	62–175	11	83	34–156	0.0452
	12 months	11	82	54–164	4	111	38–184	0.9091

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	21	75			21	24		
	3 months	19	89	2	200	16	21	4	37
	6 months	18	95	3	192	13	101	8	102
	12 months	12	84	8	146	4	111	14	202
Children's stool filtrates	4 days	21	75			21	42		
	3 months	19	78	2	168	16	46	4	14
	6 months	18	108	3	244	13	101	8	20
	12 months	12	82	8	89	4	165	15	183

Results

All results are given in tables showing the numbers of samples, cytokine concentrations in pg/ml and statistical significance of differences between 'allergic' and 'healthy' groups. Some analyses are missing because it was not always possible to obtain all samples and in sufficient amounts during the 1 yr monitoring of the children. It is very difficult or impossible to compare statistically the results of breastfed and non-breastfed children because the numbers of non-breastfed children are small with the exception of 1-year-old children of allergic mothers, where on the contrary, there are only six breastfed children and data are from four of them.

IL-4

Allergic mothers and their children showed higher IL-4 levels than healthy mothers and their children (Table 2). The IL-4 levels in the sera of allergic mothers were highly significantly higher than in healthy mothers ($p = 0.0006$). The difference between the levels of IL-4 in the children's cord blood was barely significant ($p = 0.06$) but the IL-4 level in the blood of children of

allergic mothers started to rise significantly immediately after birth, in contrast to children from healthy mothers. This rise ceased after 3 months and was followed by a slow decline so that no difference was seen between the children of allergic and healthy mothers at 1 yr of age. The IL-4 level in the colostrum of allergic mothers was mostly higher than in healthy mothers but the difference was not significant in view of a large individual variability. In the milk of allergic mothers, the IL-4 rose, peaking 3 months after delivery, and then it dropped. A similar dynamics was found in the IL-4 level in the stool of children of allergic mothers, the level in 1-year-old children being again low. In the healthy group the IL-4 levels in all tested fluids were low and did not perceptibly change during the first year of life. There were no remarkable differences in IL-4 concentrations between breastfed and non-breastfed children in the two groups under investigation.

IL-5 and IL-6

No differences were found between the two groups in IL-5 and IL-6 levels (Tables 3 and 4).

Table 5. IL-10 levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	21	4,116	24–449	21	174	135–338	0.3266
	4 days	21	61	28–391	21	258	130–523	0.0498
	3 months	21	303	49–495	20	429	182–829	0.1088
	6 months	21	386	108–660	21	239	49–439	0.2178
	12 months	20	295	122–423	18	327	137–1,978	0.2994
Children's stool filtrates	4 days	21	97	20–208	21	199	151–401	0.0169
	3 months	21	284	34–605	20	342	193–667	0.1967
	6 months	21	338	187–722	21	295	31–610	0.1996
	12 months	20	411	166–644	19	592	212–1,156	0.3612
Mother's serum	Time of delivery	21	154	21–238	20	301	167–728	0.0233
Mother's milk	4 days	21	27	16–247	20	197	46–327	0.0720
	3 months	18	75	18–335	14	400	205–524	0.0053
	6 months	16	246	148–525	11	27	13–280	0.0218
	12 months	12	429	133–722	4	217	84–370	0.2030

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	21	61			21	258		
	3 months	19	303	2	294	16	586	4	269
	6 months	18	403	3	186	13	239	8	175
	12 months	12	282	8	295	4	187	14	360
Children's stool filtrates	4 days	21	97			21	199		
	3 months	19	333	2	0	16	538	4	192
	6 months	18	332	3	824	13	343	8	137
	12 months	12	411	8	475	4	556	15	592

The levels were low in all tested fluids and did not change much during the first year of life.

IL-10

The IL-10 levels in the sera of allergic mothers at the time of delivery were higher than in healthy mothers ($p = 0.02$) (Table 5). No differences between the two groups were found in cord serum but 4 days after birth, serum IL-10 was higher in children of allergic mothers than in children of healthy mothers on the border of significance. The same was true for breastfed children at the age of 3 months ($p = 0.05$). Children of allergic mothers displayed slightly higher IL-10 levels in the stool on day 4 after birth ($p = 0.02$), differences between normal and allergic colostrum being less significant. In 1-year-old children, the IL-10 levels in sera and stool corresponded to those of the healthy counterparts. Allergic mothers had substantially higher IL-10 concentration in 3 months milk ($p = 0.005$), which decreased sharply until month 6 and was then lower than in the milk of healthy mothers ($p = 0.02$).

IL-13

There are relatively high levels of IL-13 in cord blood, which decrease sharply till the fourth day after delivery (Table 6). The decrease could reflect the largely placental origin of newborn's IL-13. IL-13 concentration in stool filtrates, which reflects roughly IL-13 concentrations in maternal milk, was low, the only significant difference between control and allergic group being found in month 6 – the concentrations in allergic group were lower ($p = 0.015$); the significance was high when only breastfed children were compared ($p = 0.005$).

IFN- γ

No statistically significant differences were proved between the two groups at birth (Table 7). Although the IFN- γ levels in the cord blood of the healthy group were usually high while those in the allergic group were mostly low, the differences could not be evaluated as significant because of large individual variations. High IFN- γ concentration in cord blood of the healthy

Table 6. IL-13 levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	21	916	151–3,027	21	2,634	208–3,954	0.2225
	4 days	19	471	22–1,614	21	412	152–588	0.6163
	3 months	21	861	309–2,524	19	1,409	371–3,530	0.5334
	6 months	21	919	218–3,225	21	1,499	295–4,686	0.5800
	12 months	20	613	123–1,862	16	1,664	695–4,758	0.0827
Children's stool filtrates	4 days	21	507	12–1,006	21	61	0–1,111	0.2614
	3 months	21	75	16–636	20	176	1–1,680	0.9688
	6 months	21	183	84–1,769	21	61	1–309	0.0152
	12 months	20	60	3–258	19	273	65–3,352	0.0698
Mother's serum	Time of delivery	21	1,019	321–2,118	20	504	129–1,998	0.3154
Mother's milk	4 days	21	151	0–1,935	20	99	0–1,038	0.8141
	3 months	18	264	55–488	15	342	0–1,708	0.8002
	6 months	16	181	93–1,473	12	93	0–283	0.0818
	12 months	12	270	87–722	4	1,359	37–3,291	0.9034

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	19	471			21	412		
	3 months	19	861	2	1,970	16	1,200	3	2,612
	6 months	18	582	3	3,187	13	1,693	8	1,266
	12 months	12	613	8	587	4	630	12	2,534
Children's stool filtrates	4 days	21	507			21	61		
	3 months	19	121	2	0	16	281	4	5
	6 months	18	145	3	1,456	13	18	8	187
	12 months	12	106	8	46	4	206	15	390

group decreased sharply till day 4, pointing to high placental production of IFN- γ in the healthy group. The concentrations on day 4 are practically equal in both groups. The most conspicuous differences in sera and stool appeared at 1 yr of age. In the healthy group, the IFN- γ in sera and stool markedly dropped whereas in the allergic group it displayed higher levels (sera – allergic vs. healthy group $p = 0.0015$; stool – allergic vs. healthy group $p = 0.002$). Similar mutual relationship between healthy and allergic groups is in both breastfed and non-breastfed children. No sizable differences were found between the mother's blood levels in the healthy and the allergic group, and no significant differences were documented in colostrum and milk.

TGF- β

No differences were observed between the two groups in the levels of this cytokine (Table 8). The very high TGF- β levels in children's blood remained high throughout the whole monitored period. TGF- β in stool gradually increased from birth, reaching a high level in 1-year-old children.

The levels of TGF- β in colostrum and milk were relatively low although the levels in mother's blood were high.

Discussion

This study aims at comparing the post-natal development of cytokine levels in children of healthy and allergic mothers and determines the proportion of individual cytokines in colostrum and milk of healthy and allergic mothers. The work is the first part of a long-term study, which will comprise prolonged (several years) clinical monitoring of the children in an effort to show if the levels of some cytokines in the early childhood could be used for predicting the appearance and course of later allergies. The results presented here concern the time interval from birth until 1 year of age. During this period, none of the children of either healthy or allergic mothers showed any symptoms of allergic disease. We shall be able to evaluate the predictive value of our present results only later during the study.

The post-natal development of cytokine levels showed differences between children of allergic mothers on the one hand and children of healthy

Table 7. IFN- γ levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	19	868	2–2,291	21	61	46–450	0.2129
	4 days	20	160	5–1,706	20	163	50–888	0.9461
	3 months	20	243	47–1,082	20	128	41–545	0.3721
	6 months	19	171	45–1,529	21	104	12–1,084	0.6746
	12 months	20	40	6–50	18	221	42–3,196	0.0015
Children's stool filtrates	4 days	21	276	26–1,569	20	77	58–445	0.4113
	3 months	21	461	98–1,247	20	252	51–664	0.4264
	6 months	21	647	82–2,436	21	23	1–740	0.0123
	12 months	20	37	5–49	19	415	29–2,215	0.0024
Mother's serum	Time of delivery	21	237	3–870	20	90	52–519	0.9688
Mother's milk	4 days	21	708	8–2,228	20	70	49–436	0.5750
	3 months	18	175	3–792	13	225	53–1,396	0.3367
	6 months	16	72	18–356	11	14	1–343	0.2267
	12 months	11	49	12–87	4	240	17–911	0.5569

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	20	160			20	163		
	3 months	18	316	2	138	16	82	4	502
	6 months	17	171	2	176	13	98	8	210
	12 months	12	47	8	6	4	78	14	221
Children's stool filtrates	4 days	21	276			20	77		
	3 months	19	722	2	187	16	242	4	333
	6 months	18	497	3	3,010	13	23	8	55
	12 months	12	47	8	19	4	1,196	15	356

mothers on the other. Despite the individual variations within the groups, some of the differences between them were highly significant. A number of authors have studied cytokines in small children and in milk of healthy and allergic mothers (11, 13–17). The results however were not unambiguous and no study covered at the same time mother's milk, child's blood and stool filtrates for an extended time period and with inclusion of a larger number of cytokines. Milk cytokines are assumed to exert regulatory action on the newborn's immune system (13, 18) but the results of the studies have been hampered by large individual variability.

We detected the most significant differences between healthy and allergic groups in IL-4, which is fully in accord with the knowledge of the role of this cytokine in allergic reactions. Increased IL-4 levels in the early post-natal period have been reported to precede the later appearance of atopic diseases (14). Less consistent but significant differences were found in IL-10, IL-13 and IFN- γ .

There are higher concentrations of IL-4 and IL-10 in the blood of allergic mothers reflecting

Th2 pre-dominance in allergy. No significant differences were found in colostrum of healthy and allergic mothers but the tendency to higher levels of IL-4 and IL-10 in allergic colostrum and IFN- γ in normal colostrum is evident. The situation is different in mature milk – there is a higher IL-4 concentration and different dynamics of IL-10 in allergic mothers. The question of the anti-allergic action of maternal colostrum and milk is still not clear, in particular as concerns the colostrum and milk of allergic mothers. The higher concentration of IL-4 in allergic milk could act pro-allergically but it is a question if it is of any importance in children with high systemic IL-4 levels. It is difficult to evaluate the effect of breastfeeding because the numbers of non-breastfed children are very low.

Cord bloods of all children tested were comparable but differences between healthy and allergic groups start to be evident already on the fourth day after birth and then during the first year of life. The levels of cytokines in cord blood are relatively high; these cytokines are formed pre-natally for the most part by trophoblast cells (17). For this reason, the level of some

Table 8. TGF- β levels in mothers and their children (pg/ml)

Sample	Time after delivery	Healthy mothers and their children			Allergic mothers and their children			p
		Number of children	Median	25%–75% percentile	Number of children	Median	25%–75% percentile	
Children's serum	Cord blood	21	70,656	44,529–1,12,847	20	63,863	35,138–1,14,335	0.7444
	4 days	21	72,105	43,730–1,39,571	18	86,006	38,835–1,26,576	0.8547
	3 months	21	1,14,570	69,717–1,56,000	19	85,806	39,237–1,29,105	0.1294
	6 months	21	49,893	34,059–1,08,758	21	54,153	33,018–1,03,533	0.9599
	12 months	19	25,854	5,682–57,126	18	38,198	12,372–74,556	0.2674
Children's stool filtrates	4 days	20	578	431–1,515	20	553	382–1,021	0.6750
	3 months	21	519	284–1,348	19	876	355–10,515	0.2442
	6 months	21	604	159–6,842	21	1,289	103–7,689	0.3924
	12 months	19	2,829	273–26,671	19	2,316	207–2,85,600	0.5993
Mother's serum	Time of delivery	21	51,357	25,998–80,874	19	67,476	28,620–90,090	0.4165
Mother's milk	4 days	21	471	219–821	18	300	70–734	0.3749
	3 months	18	229	43–387	13	239	93–522	0.7037
	6 months	16	177	47–292	11	189	9–612	0.7113
	12 months	10	213	77–1,151	4	432	200–505	0.8392

Sample	Time after delivery	Healthy mothers and their children				Allergic mothers and their children			
		Breastfed		Non-breastfed		Breastfed		Non-breastfed	
		Nr.	Median	Nr.	Median	Nr.	Median	Nr.	Median
Children's serum	4 days	21	72,105			18	86,006		
	3 months	19	1,12,032	2	1,50,762	15	60,837	4	1,08,317
	6 months	18	45,918	3	77,625	13	49,892	8	80,759
	12 months	11	40,842	8	7,419	4	29,466	14	49,071
Children's stool filtrates	4 days	20	578			20	553		
	3 months	19	492	2	5,786	15	876	4	3,431
	6 months	18	538	3	1,005	13	1,750	8	1,110
	12 months	12	568	8	7,187	4	1,226	15	5,159

cytokines after birth first decreases before it starts to rise again slowly due to the production in the newborn's organism. In children of healthy mothers, the cytokines that decrease are IL-4, IL-10, IL-13 and IFN- γ , the levels of IL-4 stay low throughout the first year of life. In children of allergic mothers the initial drop with subsequent rise occurs only with IL-13. In contrast, IL-4, IL-10 and IFN- γ do not exhibit the initial decrease but their levels start to rise immediately after birth.

Higher IL-4 in children's sera and stool, higher IL-10 in sera and stool 4 days after delivery and lower IL-13 and IFN- γ in 6-month stool are characteristic for allergic group. Differences in colostrum and milk did not correspond in all cases to the cytokine levels in the intestine, which could be caused by the active local cytokine synthesis. At the age of 1 yr, children's sera and stool filtrates of both groups are again comparable with the exception of IFN- γ , whose levels are much higher in the allergic group than in the healthy one. This is difficult to explain – it could suggest a compensatory increase of intestinal production of this anti-allergic cytokine in the group at a higher allergy risk. Generally, the

interpretation of the results is not easy because of large individual differences within the groups. If later some children get allergic it would be possible to see the possible correlation with some irregularities in cytokine spectra.

TGF- β undoubtedly plays an important part in the regulation of mucosal immune response (10) and participates in the induction of mucosal tolerance and IgA formation (10, 12). In children, milk TGF- β has been stated to act as a suppressive cytokine which aids in the development of mucosal tolerance (8, 15, 19, 20); children breast-fed by colostrum with low TGF- β content have been suspected to be predisposed to allergy (16). However, we assume that milk TGF- β is very probably not decisive for the development of tolerance because the child itself produces large quantities of TGF- β , its level in the child's intestinal contents being higher than in milk and the values being similar in suckled and non-suckled children.

The pattern of movement of levels of the cytokines examined in this study seems to indicate that the major regulatory changes in the early post-natal period could be crucial for the future immunological tuning of the organism.

This period is apparently characterized by a certain regulatory instability associated with the decision whether the responses prevalent in the future will be more of the Th1 or the Th2 type. The levels and dynamics of cytokines reflect regulatory differences between the healthy group and the group with allergic background.

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