

Relation between stressful life events, neuropeptides and cytokines: results from the LISA birth cohort study

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Stressful life events evidently have an impact on development of allergic diseases, but the mechanism linking stress to pathological changes of immune system function is still not fully understood. The aim of our study was to investigate the relationship between stressful life events, neuropeptide and cytokine concentrations in children. Within the LISApplus (Life style-Immune system-Allergy) study, blood samples from children of 6 yr of age were analysed for concentration of the neuropeptides vasoactive intestinal peptide (VIP), somatostatin (SOM), substance P (SP) and the Th1/Th2 cytokines interferon- γ (IFN- γ) and interleukin (IL)-4. Life events such as severe disease or death of a family member, unemployment or divorce of the parents were assessed with a questionnaire filled in by the parents. For 234 children, blood analysis and questionnaire data regarding life events were available. Children with separated/divorced parents showed high VIP levels and high concentrations of the Th2 cytokine IL-4 in their blood. Severe diseases and death of a family member were neither associated with neuropeptide levels nor with cytokine concentrations. Unemployment of the parents was associated with decreased IFN- γ concentrations in children's blood but not with neuropeptide levels, whereas children experiencing concomitant severe disease and death of a family member had reduced SP blood levels. The neuropeptide VIP might be a mediator between stressful life events and immune regulation contributing to the Th2 shifted immune response in children with separated/divorced parents. Unemployment of the parents was associated with immune regulation in children on the basis of a still unknown mechanism whereas reduced SP levels seem to have no effect on immune regulation.

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A growing body of research point to the direction that psycho-social stress serves as a risk factor for the development of immune system-related diseases like allergies, asthma and atopic dermatitis, especially during childhood (1–3). Moreover, clinical observations indicate that stressful life events often precede exacerbations in the symptoms of atopic diseases (4). Thus, it is hypothesized that the

immune system might be a mediator between stress and allergic diseases.

Impacts of stressful life events on immune system concerning cellular immunity (5, 6) and reduced vaccine responses (7, 8), have been observed in students around the time of examination. Other reports showed an altered immune reactivity in caregivers of dementia and Alzheimer patients as a consequence of chronic stress

(9, 10). Furthermore, it has been demonstrated that stressful life events and social support were related to cytokine production and antibody status following vaccination (11, 12) and that psychological stress affects response to immunotherapy (13) and may contribute to induction of autoimmunity (14).

As of today, however, little is known about the *in vivo* mechanism of converting stressful life events into immune cellular dysfunction. The hypothalamic–pituitary–adrenal system (HPA axis) is a feedback loop by which signals from the brain trigger the release of hormones needed to respond to stress. The stimulation of this system leads finally to the secretion of glucocorticoids and catecholamines. In addition to these classic neuromediators, which in most cases down regulate the immune system function, also neuropeptides have been proposed to have an immunological mode of action. Neuropeptides are secreted by free nerve endings of the autonomic nervous system within the central lymphoid organs and peripheral tissues where they can interact directly with immune cells via specific receptors (15, 16). As an example, T lymphocytes and dendritic cells express receptors for the neuropeptides substance P (SP), somatostatin (SOM) and vasoactive intestinal peptide (VIP). It has been shown that these neuropeptides can directly interact with T cells and may alter the Th1/Th2 balance (16–18). Recent reports describing the release of these neuropeptides under stressful conditions (19, 20), inspired our study to investigate whether a relation between these neuropeptides, stressful life events and immune regulation in children exists. In the context of an epidemiological study, we analysed in blood samples of children the concentration of the neuropeptides SOM, VIP and SP and immune parameters of relevance for Th1/Th2 regulation that is interferon- γ (IFN- γ) as an indicator for Th1 reactivity and interleukin (IL)-4 as indicators for Th2 reactivity. These parameters were correlated with the occurrence of stressful life events in particular death or severe disease of a family member, separation of parents and unemployment in a population-based sample of 6-yr-old children. These life events were assessed by a standardized questionnaire.

Methods

Study design and subjects

The Life style Immune System Allergy study (LISA) was designed to investigate the influence

of life style and environmental factors on the maturation of the immune system and the atopic risk in early childhood. Three thousand and ninety-seven newborns, born between December 1997 and January 1999 in four German cities Munich, Leipzig, Wesel and Bad Honnef, were recruited. Measurements of neuropeptide and cytokine concentrations were performed only in the city of Leipzig, thus for the present investigation only data from this cohort were used. In the LISA study, only healthy term neonates of German descent were included and newborn children whose mothers suffered from autoimmune disease or infectious disorders during pregnancy were excluded. The design of LISA has been described in detail elsewhere (21). The present investigation concerns to blood and questionnaire data from children's 6th year of life. In addition, a questionnaire on psycho-social aspects of life and stressful life events was sent to the parents at the beginning of the child's month of birth together with the regular 6-yr LISA follow-up documents. Participation in the study was voluntary and informed consent was obtained from the parents of all children. The study was approved by the ethics committee of the University of Leipzig.

Blood sampling

Heparinized blood (7 ml) was obtained by venipuncture and prepared within 4 h to further analysis. For neuropeptide ELISA and cytokine measurement, heparinized blood was diluted 1:1 with RPMI1640 medium without supplements and centrifugated at 800 *g* for 5 min at 4°C. Collected cell free supernatants were stored at –80°C until analysis.

Neuropeptide ELISA

The ELISA kits for VIP, SOM, SP were purchased from Phoenix Europe (Karlsruhe, Germany) and are based on the principle of competitive enzyme immunoassay. All measurements were performed in duplicate. Concentration of neuropeptides was determined with the EASYWIN KINETICS 32 (TECAN, Crailsheim, Germany) software by interpolation using the standard curve. To correct interassay variation, a control sample was included on each plate. A correction factor was calculated as mean concentration of all control samples divided by plate concentration of control sample. Neuropeptide concentrations are given after multiplication with this calculated factor. Detection limit for each neuropeptide was 7 pg/ml.

Cytometric bead array

Concentrations of the cytokines IFN- γ and IL-4 were measured by flow cytometry using the BD CBA Human Soluble Flex Set system (Becton Dickinson, Heidelberg, Germany) according to manufactures instructions.

In brief, cytokine specific antibody-coated beads were incubated for 1 h with 25 μ l of diluted plasma samples or standard solution. Thereafter, samples were incubated with the corresponding phycoerythrin (PE)-labelled detection antibodies for 2 h. After one washing step, samples were measured by flow cytometry. Analysis of data and quantification of cytokines were performed using the FCAP ARRAY™ software (Becton Dickinson, Heidelberg, Germany) on the basis of corresponding standard curves. Finally, plasma dilution factor was accounted for. Detection limits were 3 pg/ml for all cytokines.

Assessment of exposure

Exposure to stressful life events which occurred during the first 6 yr in children's life were assessed by questionnaires based on a commonly used life event scale (22). In particular, we focused on death or severe disease within the family, i.e. parents, grand parents or siblings ('Did you or a member of your family develop a severe physical illness that lead to hospitalization or caused this person to be bed-bound for more than 10 days? Did a member of your family die?'). Furthermore, it was asked if one of the parents had been unemployed ('Have you or your spouse been unemployed for more than 3 months?') and whether parents divorced or separated ('Did you experience a separation or divorce from your partner?'). If parents reported about separation before divorce, the year of separation was taken into account for calculation of the relationship between this life event, neuropeptides and cytokines and if parents reported about more than one separation/divorce the last event was taken for analysis. All life events were obtained retrospectively during the 6-yr follow-up examination and parents were asked to state the year of their happening.

Statistical analysis

We performed descriptive statistics and tested the relation between life events, neuropeptides and cytokines. Chi-squared test was used to test the relationship between the analysed subcohort and the remaining children of the LISA cohort from the city of Leipzig. Given that blood analysis for

neuropeptides and the cytokines IL-4 and IFN- γ was performed only in the 6th year of life, we categorized the children into three groups: (i) children with reported life events not longer than 1 yr previous to blood analysis (exact time span between 5.5 and 6 yr of age, defined as 'at 6th yr of age'), (ii) with life events longer than 1 yr ago prior to blood analysis (exact time span between 0 and 5.5 yr of age, defined as 'before 6 yr of age') and (iii) children without any life event at any time (defined as 'no'). Data for life events, neuropeptide and cytokine concentrations are presented as median with 25th to 75th percentiles. The non-parametric Mann-Whitney *U*-test was applied for the crude analysis of relation between life events, neuropeptide and cytokine concentrations that were not normally distributed. *p*-values from the *U*-test below 0.05 were considered as statistically significant. After logarithmic transformation of neuropeptide and cytokine concentrations, the association with life events was calculated by means ratios (MR), i.e. the ratio of the geometric mean in the category of study vs. the reference category (no life events). We adjusted for gender, parental history of atopy, maternal school education and presence of siblings. Cytokine and neuropeptide concentrations below the limit of detection were assigned a value of one-half of the detection limit and were included in the statistical analysis. Statistical analysis was performed with STATISTICA for Windows Version 7.0 (Statsoft (Europe), Hamburg, Germany).

Results

Subjects and exposure

At the age of six, 565 children participated in Leipzig in this study. For 324 of these children, parents agreed with blood collection and neuropeptides and Th1/Th2 cytokines have been measured in 321 of the samples.

Of the the LISA study participants from the city of Leipzig, a total of 479 families answered the life-event questionnaire. The majority of questionnaires were completed by the mothers (96%). Two hundred and thirty-four children fulfilled the criteria to have a complete life event questionnaire and data from blood analysis, thus only data from these children are shown.

Basic characteristics of the study population are given in Table 1. The parental history of atopy (single and double) was 42.7%. Level of maternal education was intermediate with 51% of mothers having had 10 yr or more of school education. Reported life events within the 6 yr of life were mostly disease (44.8%) or unemployment (29.9%)

Table 1. Characteristics of analysed subcohort (N = 234) and remaining children of overall LISA cohort from Leipzig (because of missing information for some characteristics, number of subjects varies between 322 and 331)

Variables	Analysed subcohort n (%) N = 234	Remaining subjects of LISA cohort n (%) N = 331	p*
Gender			
Male	119 (50.8)	155 (46.8)	0.567
Female	115 (49.1)	176 (53.2)	
Parental history of atopy			
None	134 (57.3)	187 (56.5)	0.415
Single positive	76 (32.5)	114 (34.4)	
Double positive	24 (10.2)	30 (9.1)	
Maternal school education			
Low	4 (1.7)	16 (4.9)	0.941
Middle	119 (50.8)	167 (51.9)	
High	111 (47.4)	139 (43.2)	
Presence of siblings			
One ore more siblings	187 (79.9)	239 (73.8)	0.306
No siblings	47 (20.1)	85 (26.2)	

*p-value from the chi-squared test for cross table relationship.

High, 12 yr of schooling or more 'Fach-/hochschulreife'; intermediate, 10 yr of schooling 'Mittlere Reife'; low, 9 yr of schooling or less 'Hauptschulabschluss'.

and less cases of death (15.8%) and divorce or separation of the parents (10.3%), Table 2. Regarding to this covariates, there was no difference between the analysed subcohort (n = 234) and the remaining children from the LISA cohort from Leipzig (n = 331). Only few children experienced a multiple exposure to stressful life events during the 6th year of life. At that time, four children experienced severe disease of a family member together with death of a family member and one child experienced concomitant unemployment and disease of a family member. The group of children with recent separated/divorced

Table 2. Frequency of life events for the analysed subcohort (N = 234) of 6-yr-old children from the LISA study group from the city of Leipzig

Variables	Frequency n (%)
No life event at all	80 (34.2)
No	
Death of a family member	197 (84.2)
Severe disease of a family member	129 (55.1)
Unemployment	164 (70.1)
Divorce/separation	210 (89.7)
Life event before 6 yr of age	
Death of a family member	32 (13.7)
Severe disease of a family member	82 (35.0)
Unemployment	55 (23.5)
Divorce/separation	18 (7.7)
Life event at 6 yr of age	
Death of a family member	5 (2.1)
Severe disease of a family member	23 (9.8)
Unemployment	15 (6.4)
Divorce/separation	6 (2.6)

parents (n = 6) experienced no other stressful life events at the age of 6.

Neuropeptides

The relation between life events within the year prior to blood analysis (children at 6 yr of age) and neuropeptide concentration is given in Table 3 and Fig. 1.

When parents reported about divorce or separation within 1 yr previous to children's blood analysis, mean blood concentration of the neuropeptide VIP (302 pg/ml) in these children was significantly higher than in children without separated/divorced parents (188.6 pg/ml; $p = 0.005$ from the Mann–Whitney *U*-test). Neither SOM nor SP blood levels were associated with divorce/separation of the parents. Even after adjustment with the possible confounders gender, parental history of atopy, maternal school education and presence of siblings, the association between VIP and separation/divorce of the parents remained significant (adjusted MR 1.72; 95% CI 1.02–2.88).

Death of a family member within 1 yr previous to children's blood analysis, was significantly ($p = 0.04$) correlated with decreased VIP and SP values in the blood of affected children compared with those without this life event at any time, Table 3. However this association was not significant after adjustment to the possible confounders. Children experiencing concomitant severe disease and death of a family member within the 6th year of life (n = 4) had significantly decreased blood concentrations of SP (115.7 pg/ml) compared with children without stressful life events (217.8 pg/ml; $p = 0.008$, Mann–Whitney *U*-Test). Even after adjustment with the possible confounders, these association remained significant (adjusted mean ratio 0.47; 95% CI 0.24–0.91). No other associations of neuropeptide concentrations and exposure to multiple stressful life events were observed. The separate exposure to unemployment and severe disease of a family member were not associated with neuropeptide concentrations, neither on crude data (Table 3) nor after adjustment with possible confounders (Fig. 1). When life events occurred longer than 1 yr ago prior to blood analysis, no associations to neuropeptide concentrations have been found (Table 4 and Fig. 2).

Cytokines

Life events such as death or severe illness of a family member were not associated with changes

Table 3. Relation between life events (within the year prior to blood analysis) and concentrations of neuropeptides and cytokines (crude data) in blood samples from 6-yr-old children (N = 234). Shown are medians and the 25th to 75th percentiles

[pg/ml]	No (n = 80)	Death of a family member		Severe disease of a family member		Unemployment of parents		Divorce/separation of parents	
		n = 5	p*	n = 23	p*	n = 15	p*	n = 6	p*
VIP	188.6 (141.7–243.4)	125.2 (104.2–125.4)	0.044	195.3 (125.4–249.4)	0.874	175.2 (135–244.2)	0.517	302 (206.5–366.5)	0.005
SOM	31.8 (23.5–44)	25.6 (17.9–49)	0.837	24.2 (17.2–55.7)	0.547	35.2 (24.8–53.4)	0.690	29.6 (24.7–35.3)	0.959
SP	217.8 (141.4–260.6)	124.2 (107.4–129.8)	0.042	166.1 (121.6–261.9)	0.277	235.3 (111.2–279.1)	0.951	184.6 (173.1–228.2)	0.834
IL-4	5.7 (1.5–9)	5.9 (5.8–7.7)	0.741	5.4 (1.5–8.9)	0.771	5.8 (1.5–9.2)	0.831	11.8 (7.3–25.6)	0.023
IFN- γ	8.9 (1.5–13.9)	8.2 (7.2–10.8)	0.841	7.1 (1.5–12.1)	0.731	1.5† (1.5–12.6)	0.126	5.8 (1.5–14.7)	0.917

*p-values from the Mann–Whitney *U*-test compared with the group without corresponding life events.

Limit of detection: 7 pg/ml for all neuropeptides and 3 pg/ml for all cytokines.

†35% of values were below the detection limit.

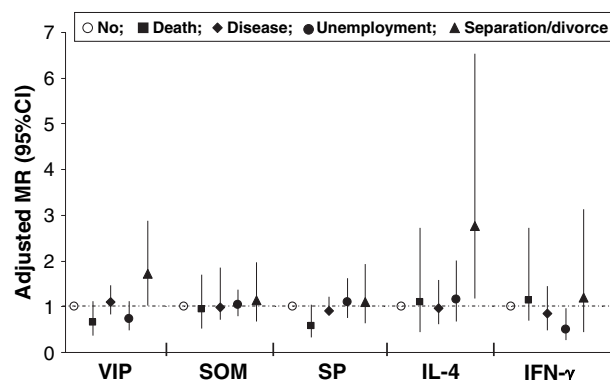


Fig. 1. Relation between life events within the year prior to blood analysis (at 6 yr of age), neuropeptide and cytokine concentrations in blood samples of 6-yr-old children. Data are presented as means ratios (MR) and 95% confidence intervals for VIP, SOM, SP, IL-4 and IFN- γ adjusted for gender, parental history of atopy, maternal school education and presence of siblings.

in cytokine concentrations in affected children compared with infants without these life events. In contrast, divorce/separation of the parents was significantly ($p = 0.023$ from the Mann–Whitney *U*-test) associated with increased IL-4

levels (11.8 pg/ml) in children exposed to this life event in the 6th year of life compared with those without this experience (5.7 pg/ml, Table 3). After adjustment to the possible confounders, these relationships remained significant (adjusted MR 2.77; 95% CI 1.17–6.53). Similar to the results observed for the neuropeptide VIP, IL-4 concentrations were not different between children having separated parents longer than a year ago prior to blood analysis and children without this life event experience (Table 4, Fig. 2). For the Th1 cytokine IFN- γ , changes in concentrations have been observed only in children with unemployed parents. Here, a significant association to reduced IFN- γ levels was found after adjustment to gender, parental history of atopy, maternal school education and presence of siblings (adjusted MR 0.51; 95% CI 0.27–0.97, Fig. 1). No associations between life events occurring longer than 1 yr ago prior to blood analysis and IFN- γ concentrations were found (Table 4, Fig. 2). We found no difference in blood cytokine concentrations comparing children exposed to multiple stressful life events and non-exposed children.

Table 4. Relation between life events (longer than 1 yr ago prior to blood analysis) and concentrations of neuropeptides and cytokines (crude data) in blood samples from 6-yr-old children (N = 234). Shown are medians and the 25th to 75th percentiles

[pg/ml]	No (n = 80)	Death of a family member		Severe disease of a family member		Unemployment of parents		Divorce/separation of parents	
		n = 32	p*	n = 82	p*	n = 55	p*	n = 18	p*
VIP	188.6 (141.7–243.4)	201.3 (136.1–292.4)	0.228	215.5 (155.4–256.4)	0.107	195.3 (134.7–264.9)	0.324	195.5 (138.7–279.1)	0.523
SOM	31.8 (23.5–44)	28.9 (24.3–40.1)	0.553	29.2 (19.8–40)	0.202	28.4 (18.7–38.5)	0.203	31.5 (22–53.4)	0.963
SP	217.8 (141.4–260.6)	204.2 (129.2–248.1)	0.395	201.7 (146.2–248.5)	0.442	206.6 (146.5–265.8)	0.986	214.5 (145.9–297.2)	0.847
IL-4	5.7 (1.5–9)	5.7 (1.5–7.3)	0.642	3.1 (1.5–8)	0.515	6.6 (1.5–10.1)	0.216	1.5 (1.5–8.9)	0.438
IFN- γ	8.9 (1.5–13.9)	5.8 (1.5–11.2)	0.244	6.7 (1.5–13.2)	0.513	7.2 (1.5–12.1)	0.661	7.2 (1.5–13)	0.693

*p-values from the Mann–Whitney *U*-test compared with the group without corresponding life events.

Limit of detection: 7 pg/ml for all neuropeptides and 3 pg/ml for all cytokines.

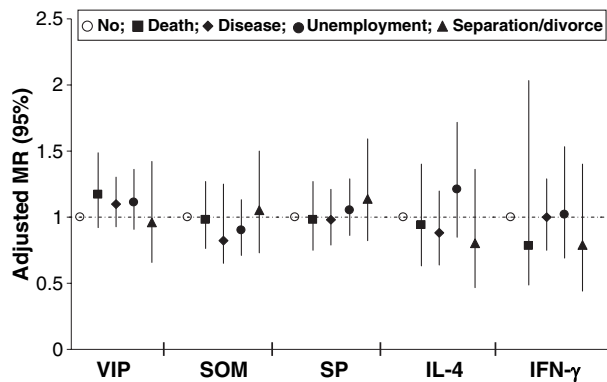


Fig. 2. Relation between life events longer than 1 yr ago prior to blood analysis (before 6 yr of age), neuropeptide and cytokine concentrations in blood samples of 6-yr-old children. Data are presented as means ratios (MR) and 95% confidence intervals for VIP, SOM, SP, IL-4 and IFN- γ adjusted for gender, parental history of atopy, maternal school education and presence of siblings.

Discussion

The aim of this study was to investigate the relationship between stressful life events and neuropeptide and Th1/Th2 cytokine blood concentrations in children who had experienced life events with a great psycho-social impact.

In the Leipzig subset of the LISA study, we found an increased blood concentration of the neuropeptide VIP in children whose parents separated or divorced not longer than a year ago prior to children's blood analysis. Furthermore, these children had increased levels of the Th2 cytokine IL-4 compared with non-exposed children. Separation or divorce of the parents dated back longer than 1 yr showed neither an effect on neuropeptide levels nor on immune parameters.

Another potential stressor which was analysed with respect to immune regulation was unemployment. In the study population of Leipzig, a high proportion, of at least one of the parents, was unemployed (42.7%). This life event might not be perceived as stressful by the children as it was not associated with increased neuropeptide levels. One explanation for this finding could be the fact that unemployment causes only stress for the parents but not for their children. But, unemployment may have an effect on immune regulation in affected children via other mechanisms, as the Th1 cytokine IFN- γ was reduced in children with unemployment in their families. So far, we are not able to further specify these mechanisms.

Taken as a single exposure, severe disease or death of a family member seems to have no relationship to neuropeptide levels and the anal-

ysed immune parameters. Thus, it can be suggested that these life events are less relevant for children in terms of stress reactions. However, when these life events were experienced together recently, these children had reduced blood concentrations of SP in comparison with unaffected children but no alterations at cytokine levels.

The *in vivo* relevance of SP for immune modulation is not yet clear. In our previous investigation, we found that SP correlates with high blood IFN- γ levels and with low concentrations of the transcription factor GATA3 and was not associated to allergic sensitization of children (23). Thus, we concluded that SP might have a rather protective role in allergy development. In contrast, Kimata (2003) found out that playing video games and exposure to frequently ringing mobile phones enhanced allergic responses with a concomitant increased release of SP and skewing of the cytokine pattern toward Th2 type in patients with atopic dermatitis (20). However, as we found reduced levels of SP in relation to multiple stressful life events and no relation to cytokine levels in the present investigation, it is difficult to make conclusions for the possible *in vivo* role of SP. In addition, because of the low number of cases this result has to be interpreted with caution.

Our data support results from a former investigation within the LISA study. Bockelbrink et al. (22) showed that separation/divorce of parents in the first 2 yr of life leads to an increased incidence of atopic eczema later in life of these children. It is noteworthy that from all analysed life events in our and the study by Bockelbrink et al. (22) only separation or divorce of parents had an effect on VIP level, Th2 cytokine secretion and development of atopic eczema in children. Separate exposure to other life events like death or severe illness of a family member were after adjustment neither associated with increased neuropeptide or cytokine concentrations nor with disease development in affected children. Bockelbrink et al. found even a decreased risk for atopic eczema in children having a family member with a severe illness (22). Our finding that children with concomitant exposure to severe illness or death of a family member have reduced SP blood levels points also to the direction that these life events are not stressful for the children. Therefore, we conclude from these results that separation/divorce of the parents might be the most critical life event during childhood.

This is in line with other investigations which also demonstrated that concomitant parental and personal conflicts, but no other stressful life

events, increased the risk of asthma in affected children (3). Furthermore, a significant association between separation anxiety disorders and increased risk for atopy in exposed children has been found (24). Thus, it is evident that separation of parents may cause stress in the child and thereby leading to the release of stress mediators, which affect immune regulation.

Recently, a direct relation of VIP and other neuropeptides to stress has been demonstrated in humans as well as in animal models. Measurements in human individuals with motion-sickness before and after a parabolic flight revealed increased VIP levels after the flight (25). Moreover, in a clinical trial investigating the link between perioperative stress and stress markers, it has been found that level of VIP increased with plasma levels of the 'typical stress hormones' cortisol and catecholamines (26). Furthermore, exposure to frequently ringing mobile phones or computer-induced stress significantly enhanced plasma levels of VIP with concomitant increase of Th2 cytokines and allergen-specific T-cell responses in patients with atopic dermatitis (19, 20). All these results support the idea that an induction of VIP with subsequent Th2 bias is evident in acute stress situations. Our data reported here provide evidence that this is valid for chronic stress situations as well, which might be caused by certain stressful life events.

On cellular basis, *in vitro* and *in vivo* mouse studies indicate that VIP regulates Th1/Th2 balance. Macrophages and dendritic cells treated with VIP gain the ability to induce Th2-type cytokines (IL-4, IL-5) and inhibit Th1-type cytokines (IFN- γ , IL-2) in antigen-primed CD4 T cells (27). In concordance with these results, *in vivo* administration of VIP in immunized mice resulted in decreased numbers of Th1 and increased numbers of Th2 cells (28, 29). In addition, based on data from the same cohort we have recently reported that VIP levels in the blood of children correlate with higher sensitization frequencies to food and inhalative allergens (23) indicating that VIP is not only related with the development of a Th2-primed cellular immune response but also with the subsequent development of allergic diseases.

It is noteworthy that the relation between a stressful life event, neuropeptide blood levels and Th1/Th2 balance can only be observed close to the respective life event. Separation/divorce of parents which had occurred longer than 1 yr ago prior to blood analysis had no association to neuropeptide or cytokine levels in children's blood. Obviously, there is no long-lasting effect of this life event on neuropeptide release and

immune regulation. However, during the life period in which, mediated by VIP, the T-cell reactivity is shifted toward Th2, allergic sensitizations might be developed with a higher probability. The association between VIP blood levels and allergic sensitizations observed within the LISA study (23) may support this hypothesis. On the basis of a once-established IgE response, allergic diseases like eczema may occur later in life.

The strength of this study is that for the first time stressful life events were evaluated in a setting of a large birth cohort with the assessment of immune markers and neuropeptides. Thus, the study gives a link between questionnaires-based data, stress response and immune regulation. The obtained data support earlier published results from the same cohort (22) regarding the effect of separation/divorce of parents and development of allergic diseases in affected children.

The strongest limitation of this study is the low case number of affected children. The obtained results therefore have to be interpreted with caution, but nevertheless they give orientation and encourage for further studies.

In conclusion, the present epidemiological data show that divorce or separation of parents is associated with increased VIP levels in children's blood and with a Th2 bias in cytokine secretion. Unemployment of a family member is only associated with reduced IFN- γ levels without relation to stress markers and a concomitant experience of severe diseases and death of a family member is associated with reduced SP levels in children's blood. Hereby, we give a possible explanation for the mechanism which underlies enhanced risk for allergy development in response to stressful life events, at least for separation/divorce of the parents. However, further response pathways could also be involved and an examination of a broader range of cytokines and neuropeptides could be useful to delineate the true complexity of the development of pathological immune responses in association with stressful life events.

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