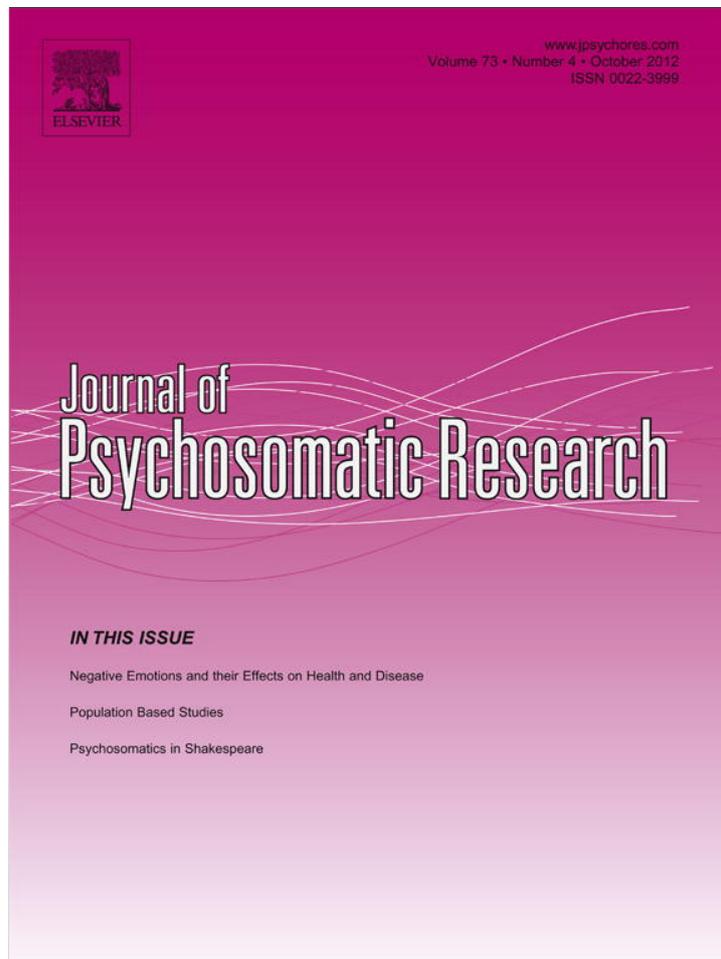


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Increased number of activated T cells in lymphocyte subsets of maltreated children: Data from a pilot study

H. Bielas^{a,b,*}, A. Jud^a, U. Lips^b, J. Reichenbach^c, M.A. Landolt^{a,d}

^a Department of Psychosomatics and Psychiatry, University Children's Hospital Zurich, Zurich, Switzerland

^b Child Protection Team, University Children's Hospital Zurich, Zurich, Switzerland

^c Division of Immunology/Haematology/BMT, University Children's Hospital Zurich, Zurich, Switzerland

^d Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland

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ABSTRACT

Objective: Maltreatment in childhood has been related to enduring changes in the immune system of adults, such as increased cell-mediated immune response.

Purpose: Due to the lack of data in children, this study examined lymphocyte subset numbers and distribution during youth.

Methods: In 27 cases of 42 healthy but maltreated children, fully participating at follow-up 1–3 years after the intervention of child protection team, and 19 cases of previously matched controls, analysis of blood samples by fluorescent activated cell sorter was consented.

Results: With regard to age references, total lymphocyte counts were aberrant in maltreated children but not in controls. When compared to controls, the percentages and absolute numbers of activated (HLA-DR+) CD4+ helper and CD8+ cytotoxic T cells were significantly higher in maltreated children.

Conclusions: According to the typical distribution of HLA-DR+ cells we assumed an increased stimulated cell-mediated immune function in maltreated children.

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Introduction

Maltreatment in childhood appears to be associated with enduring changes in the nervous, endocrine, and immune systems [1]. It can negatively affect the long-term cognitive, psychosocial, and somatic development of children [2]. Traumatic stress resulting from child maltreatment is known to alter the hormonal response of the hypothalamic-pituitary-adrenal (HPA)-axis in children [3–6] and is associated with subsequent chronic hyper-arousal in posttraumatic stress disorder (PTSD), depression, and inflammatory diseases such as irritable bowel syndrome [7,8]. However, a prolonged period of hyperactivity of the HPA-axis due to chronic stress may lead to the phenomenon of hypocortisolism [9]. Both, hyper- and hypocortisolism impact the production of cytokines and balance of helper T cell response that may explain the susceptibility to atopic or allergic and autoimmune diseases [10]. In a retrospective cohort study of 15,357

adults, the total number of adverse childhood experiences (ACEs; e.g., childhood physical, emotional, or sexual abuse; witnessing domestic violence), as a measure of cumulative childhood stress, correlated with the first hospitalizations for autoimmune diseases [11].

Only recently, it has been shown that levels of C-reactive protein (CRP) in 12-year old children were elevated if they had been exposed to physical abuse and experiencing current depression. These group differences could not be explained by potential confounders, such as family socio-economic circumstances, obesity, or body temperature [12]. According to a twin study, increasing levels of early trauma are still positively related to CRP levels in adults [13], which has been found even 20 years after maltreatment [14].

On the cellular level, traumatic stress disturbs the fine regulation of the generation, maintenance, and functioning of lymphocytes, especially in the peripheral T cell compartment, thereby impairing the balance between immunity and peripheral tolerance [15]. No relevant study has been published with regard to dysregulated pathways of the cellular immune response in maltreated children and adolescents. Studies in adults who experienced sexual abuse in childhood [16,17] or war and torture often include PTSD diagnosis. Although they partially indicate increased activation, their results on circulating immune cells are rather mixed [18]. In war veterans e.g., comorbid and complex PTSD appear to be associated with a higher prevalence of common autoimmune diseases (e.g., rheumatoid arthritis, psoriasis, insulin-dependent diabetes,

* Corresponding author at: Department of Psychosomatics and Psychiatry, University Children's Hospital, Steinwiesstrasse 75, CH-8032 Zurich, Switzerland. Tel.: +41 44 266 76 72.

E-mail address: hannes.bielas@kispi.uzh.ch (H. Bielas).

thyroid disease); and comorbid PTSD has been related to higher T cell counts and hyper-reactive immune responses (e.g., cutaneous hypersensitivity, higher immunoglobulin-M levels,) [19].

Such controversial data leave questions about the impact of maltreatment on general health and transferability to children and adolescents. In addition, the impact of psychopathology on the course of immune responses after maltreatment has not been fully ruled out. For example, depression has been characterized by higher numbers and percentages of T cells bearing T cell activation markers, such as HLA-DR+ [20]. HLA-DR+ is constitutively expressed on professional antigen-presenting cells such as dendritic cells, B cells, and monocytes/macrophages. On T cells and NK cells the expression of HLA-DR+ is induced by cell activation, e.g. in the course of infection or by unspecific stimuli. It is also upregulated in patients with autoimmune diseases and during chronic viral infection [21].

Purpose

The lack of information regarding early changes in the immune system subsequent to maltreatment in childhood motivated the present study. Based on the above reported previous findings on alternations of child and adult immune responses related to maltreatment and reports of a dysregulated inflammatory response, such as increased morbidity of autoimmune diseases, we expected to find a relative and persistent activation of the immune system in maltreated children when compared to a control group. Therefore, we aimed to examine the distribution and activation via HLA-DR+ of blood lymphocyte subsets in maltreated children compared to healthy controls and with respect to age references.

Materials and methods

Sample

In the years 2005 and 2006, the Child Protection Team (CPT) at the University Children's Hospital Zurich visited 319 children as in- or out-patients. CPT interventions are based on decisions by an interdisciplinary group made up of social workers, nurses, physicians, and mental health professionals. The services offered are unique and dependent on the individual needs of each child. They can include counseling, social assistance, psychiatric or psychological services, and referrals to child welfare services. Subtypes of maltreatment were categorized as physical/sexual/psychological maltreatment or neglect. The CPT applied definitions of child maltreatment based on the widely accepted guidelines from a Swiss governmental expert group (Arbeitsgruppe Kindesmisshandlung, see annex, Table 1), and

exclusion criteria, such as Munchausen syndrome by proxy or unsubstantiated cases [22].

A final eligible sample of 180 children remained to contact for a follow-up interview, with the intention to analyze developmental outcomes of maltreated children in a variety of psychosocial and biological domains (Fig. 1). With regard to several demographic variables and maltreatment characteristics, the only variable found to be associated with an uneven distribution in participating compared to non-participating groups was the child's nationality [23]. Written informed consent was obtained, and all procedures were approved by the local ethics committee.

In 42 cases of maltreatment the children and parents consented to participate in a face-to-face interview with an independent psychologist at the hospital, two to four years after the referral to the CPT. Corresponding to their former in- or outpatient status, each fully participating maltreated child was matched for gender, age (± 1 year) and Swiss or foreign nationality with a control, that was treated at the University Children's Hospital. The assessment of lymphocyte subsets was part of this larger study. For a subsample of 27 cases of maltreated children and 19 cases of matched controls, caregivers agreed to the child providing a blood sample. Cases of chronic diseases or severe illnesses were excluded and upon examinations of health status no clinically symptomatic viral or bacterial infections were reported in participants. With regard to gender, age, and nationality, there was no difference between the 27 maltreated children (48% of Swiss nationality; 11 boys, 16 girls; mean age 8.7 years; SD 3.7, range 2.6–16.5) and 19 controls (46% of Swiss nationality; 8 boys, 11 girls; mean age 9.3 years, SD 3.3, range 4.6–15.7).

Measures

PTSD was measured via the UCLA CPTSD-RI [24] in children above the age of six years and by the Posttraumatic Stress Disorder Semi-Structured Interview and Observational Record for Infants and Young Children for children below the age of six years [25]. Controls who fulfilled criterion A were excluded from the study and replaced with a new participant.

The existence of depressive symptoms was evaluated via the "Depressions-Inventar fuer Kinder und Jugendliche" (DIKJ) [26], the German translation of the Children's Depression Inventory [27].

Socioeconomic status (SES) was estimated by a score based on the caregiver's information of parental education and current employment. Both were rated on a scale ranging from one to six, with one being the highest education and the highest status employment. Totaled sub-scores represent statuses, ranging from a highest status of two to a lowest status of twelve. To ease

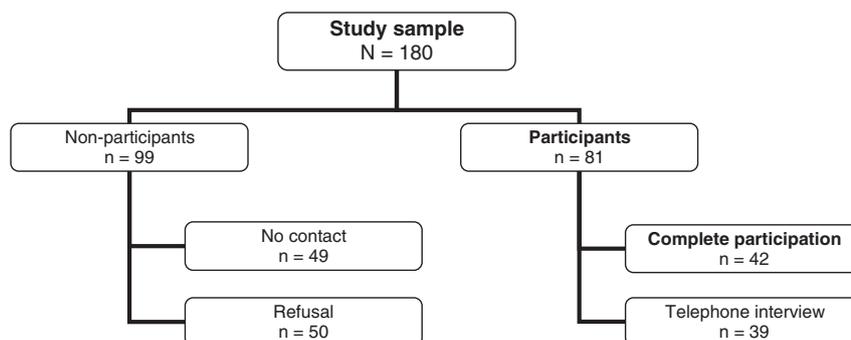


Fig. 1. Eligible sample for follow-up after CPT intervention in 2005/6.

interpretation, the score was first reversed so that higher scores represented higher SES. Then, the scores were assigned to three categories: two to five for low, six to eight for medium, and ten to twelve for high SES [28].

Lymphocyte analysis

Fluorescent activated cell sorter (FACS) analysis of lymphocyte subpopulations was performed according to standard protocols by whole blood analysis (Becton, Dickinson and company, 7 color approach, FACSDiva software) for total lymphocytes, B cells (CD19+/CD45+), NK cells (CD3-CD16+56+/CD45+), monocytes (CD14+/CD45+), recent thymic emigrants (CD31+CD45RA+/CD4+) and total T cells (CD3+/CD45+). The CD3+ T cell compartment was analysed separately for T helper cells (CD3+CD4+/CD45+) and, cytotoxic T cells (CD3+CD8+/CD45+). Moreover, the level of immune stimulation was assessed by activated helper T cells (CD4+HLA-DR+/CD3+) and cytotoxic T cells (CD8+HLA-DR+/CD3+). Age-related normal values were assessed [29,30].

Statistical analyses

Statistical analyses were conducted using SPSS for Windows software package, version 16 (SPSS Inc., Chicago, IL). As not all participants agreed to blood drawing/sampling, the number of obtained samples differed between maltreated participants and controls. Consequently, the number of matched pairs was reduced. We therefore performed chi-square tests to statistically compare the two groups (i.e., in terms of gender, age, nationality, IQ, and SES) and determine the distribution of aberrant lymphocyte counts (<10th percentile or >90th percentile; deviation from age references of the normal population; see above). All immunological parameters were analyzed for group differences using t-tests. In addition, effect sizes were computed [31]. The sample size did not allow for multiple regression analysis [32], therefore, the role of predictors could not be examined.

Results

Characteristics of the sample

In the maltreatment group, PTSD was diagnosed in eight children and rejected for 12 children. The control group was screened for criterion A of the PTSD diagnosis.

Regarding depression, in the control group, all scores were below the cutoff value ($n = 15$), whereas four maltreated children rated above and ten below the cutoff value of the DIKJ. Overall, assessable SESs ($n = 40$) were unequally distributed between the maltreated children (high: $n = 3$; medium: $n = 8$; low: $n = 13$) and the controls (high: $n = 7$; medium: $n = 9$; low: $n = 3$). As a result, the impact of these characteristics as a possible effect of maltreatment could not be analyzed.

Quantification of lymphocyte subsets

According to the Kolmogorov–Smirnov test, we did not find non-normal distributions of data for all subsets. With regard to age-specific normal ranges, in maltreated children 5 of 27 total lymphocyte counts were outside the normal range (<10th percentile or >90th percentile), whereas all controls showed absolute lymphocyte counts within age references. This difference was statistically significant ($p = 0.047$). With regard to age-specific references of lymphocyte subsets, no significant group differences between maltreated children and controls were detected in the Pearson's Chi-square tests.

As shown in Table 2, maltreated children and controls did not differ with respect to absolute counts of lymphocytes, B cells (CD19+/CD45+), total T cells (CD3+/CD45+), helper (CD3+CD4+/CD45+) and cytotoxic (CD3+CD8+/CD45+) T cell populations, or recent thymic emigrants (CD31+CD45RO+/CD4+) and monocytes (CD14+/CD45+). However, the percentage of activated helper T cells (CD4+HLA-DR+/CD3+) and activated cytotoxic T cells (CD8+HLA-DR+/CD3+) within their population was also increased in maltreated children compared to controls. Relative to mean cell counts in controls, activated helper and cytotoxic T cells were elevated by +38.4% and +70.4%, underlined by large effect sizes. The number of NK cells (CD3-CD16+56+/CD45+) was also relatively increased by +31.8% with a medium

effect size in maltreated children, which difference did not reach the level of significance (see Table 2).

Moderating variables

To ensure that these findings were not biased by gender, we differentiated the data and determined that there were no significant group differences in general lymphocyte populations with regard to age-specific normal ranges (Tables 3 and 4). Counts and percentage of activated helper T cells were increased in maltreated boys whereas in maltreated girls counts and percentage of activated cytotoxic T cells within their subpopulation have been significantly increased. The absolute number of activated helper T cells increased for maltreated girls (31.8%) and boys (50.6%) with relevant effect sizes compared to controls, reaching significance only in boys. The absolute numbers of activated cytotoxic T cells were increased in maltreated girls (80.9%) and boys (60.0%) with relevant effect sizes, reaching significance only in girls. Furthermore, in maltreated boys, the absolute number of recent thymic emigrants was significantly reduced by 33.9% (Table 4).

To eliminate the effect of possible subclinical infections that may not have been apparent upon clinical examination we excluded four participants with abnormally increased number of total lymphocytes. Results for all parameters remained unchanged, except for counts of activated helper T cells ($p = 0.08$) whereas the difference in their percentages was still significant between maltreated children and controls ($p = 0.03$).

Discussion

The current study aimed to characterize numerical changes in peripheral blood lymphocyte subsets of maltreated children. We were able to demonstrate an increased activation of these cells when compared to controls. Aberrations in total lymphocyte counts were found only for the maltreated children, even though no group differences with regard to the absolute number were detected. To date, research on maltreatment in childhood has focused on functional and structural neurobiological correlates, genetic factors, and endocrine findings [33]. Therefore, the comparison of our results with previous findings was limited, as this study is the first one to analyze lymphocyte subsets in maltreated children. Some studies in adults with PTSD have similarly reported increased lymphocyte counts [34,35], whereas others have demonstrated mixed results [36,37], limiting reliability and comparison.

Even though chronic social stress has been shown to increase T- and decrease B-lymphocyte counts in animal studies [38], our results are in line with relevant studies in adults who suffered sexual abuse during childhood that did not report differences with regard to the number of helper and cytotoxic T cells or other major T or B lymphocyte subsets [17,36]. However, PTSD symptom clusters have been found to correlate with the number of helper (CD4+) and cytotoxic (CD8+) T cells [39], whereas other studies in PTSD populations have reported either no differences in T cell counts [35] or a decrease [40].

Our main finding however is that maltreated children exhibited a marked increase in percentage and numbers of activated HLA-DR+ helper T cells and cytotoxic T cells in both boys and girls. These differences were not reflected in the total numbers of helper T cells or cytotoxic T cells and were not explained by other indicators of inflammation, such as aberrant lymphocyte counts. Although all participants were considered healthy after thorough physical examinations, we cannot formally exclude the presence of subclinical (viral) infection at the time of analysis, which may have influenced our findings.

Other potential confounders, such as SES, PTSD, and depression, were unequally distributed between the maltreatment and control groups. Moreover, their role could not be tested, due to the lack of power. The consideration of these confounders would have reduced the sample size in a way that would not have allowed appropriate statistical analysis. However, their role might be limited with regard to maltreatment, even though depression, for example, has been previously associated with increased inflammation markers, such as CRP and HLA-DR+ [20]. In a representative birth cohort of 1000 individuals

followed to age 32, the significant association of high levels of high-sensitivity (hs) CRP and depression was attenuated when the effect of childhood maltreatment was taken into account [41]. Other potential confounders, such as family socioeconomic circumstances, obesity, and body temperature, have also failed to explain the increased of CRP found in formerly physically abused 12-year-old children with current depression [12]. As an inflammatory marker, HLA-DR+ is also unspecific, but it has been shown to be upregulated in patients with autoimmune diseases, such as multiple sclerosis, and during chronic viral infections, such as HTLV-1. The relevance and function of elevated HLA-DR+ expression on T cells in supposedly healthy donors, as in our sample, remains largely unknown [21]. However, its expression on activated helper and cytotoxic T cells has been shown to be higher in joints of patients suffering from rheumatoid arthritis or osteoarthritis [42]. Because arthritis has been consistently found to be associated with PTSD [43], our findings remain to be studied with regard to pro-inflammatory activity found in PTSD-patients as well [18,44].

In addition, other indices of lymphocyte activation, such as a higher ratio of CD45RO-positive to CD45RA-positive lymphocytes (CD3CD45RO associated with memory T cells vs. CD3CD45RA associated with naive/resting T cells), have been found in adults with PTSD that was associated with a history of childhood sexual abuse [17]. In addition, the percentage of T cells expressing CD45RA, also referred to as an early activation marker, has been shown to be higher in women with PTSD following childhood maltreatment [16]. In addition to our results, these indicators of a cell-mediated immune activation suggest higher blood levels of pro-inflammatory cytokines; however, underlying mechanisms remain incompletely understood [45] and need to be studied with regard to genetic background and epigenetics [46].

Limitations

The clinical relevance of our findings, which are based on a one time-point analysis in a small sample, is uncertain. First, due to the nature of pilot studies, our study has a considerable risk of selection bias, as well as limited statistical power and assessment of potential confounders (e.g., via a multiple regression model). Moreover, the final sample of 27 maltreated children is small and hardly representative of the eligible sample treated in 2005 and 2006 ($n = 180$). Therefore, it remains unclear if increased T cell activation has been impacted by psychopathology, sub-optimal hygiene and diet, or sub-clinical inflammatory conditions. Second, because of the exploratory character of this study, we decided not to correct for multiple comparisons. In addition, we computed Cohen's *d* effect sizes (see Tables 2–4), which are independent from statistical significances and alpha-error. Third, our findings are cross-sectional and descriptive and do not establish causal links. Finally, due to ethical consid-

erations we could not assess a maltreatment group that received no CPT intervention in order to control its impact. However, to our knowledge there are no previous studies indicating negative influences of supportive interventions on the immune system. In summary, the exploratory character of this pilot study limits interpretation and suggests the need for further studies to confirm our results in greater samples that offer opportunities for multivariate analyses.

Implications

This study is the first to demonstrate a dysregulated immune system in children because of maltreatment, even at follow-up after the intervention of CPT. According to the typical distribution of HLA-DR+ T cells we assume an increased stimulated cell-mediated immune function already in maltreated children, which is in line with studies in adults, showing that cell-mediated immune activation can be associated with the experiences of childhood maltreatment. Because arthritis and other auto-immune diseases in adults have often been found to be associated with adverse childhood experiences [11] and with PTSD [43], our findings on an altered immune system in maltreated children may offer an interesting link between adverse childhood experiences and physical diseases in adulthood. However, our cross-sectional study does not provide any empirical evidence for such an association and therefore such considerations are highly speculative at this point.

With regard to the limitations of a pilot study and the need of a better understanding and evaluation of these psychobiological alterations, that from early on in life set the stage for the onset of adult diseases, further studies are warranted to validate and elucidate our findings. This would allow a more detailed analysis of confounders (e.g. psychopathology, SES) and the immune status, including cytokine production, immunoglobulin levels, vaccine-antibody response or lymphocyte proliferative response to mitogens or antigens.

Conflict of interest statement

The authors have no conflict of interest to disclose.

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Annex A

Table 1
Definitions of maltreatment types used by the CPT of the University Children's Hospital Zürich (adapted from Leeb et al., 2008)

Type of maltreatment	Definition
Physical maltreatment	Intentional use of physical force against a child that results in or has the potential to result in physical injury, includes hitting, kicking, punching, beating, stabbing, biting, pushing, shoving, throwing, pulling, dragging, dropping, shaking, strangling/choking, smothering, burning, scalding, and poisoning
Psychological maltreatment	terrorizing, isolating, restraining, confining, corrupting, exploiting, spurning, or otherwise behaving in a manner that is harmful, potentially harmful, or insensitive to the child's developmental needs or can potentially damage the child psychologically or emotionally
Neglect	Failure by the caregiver to provide basic physical and psychological needs (e.g., nutrition, hygiene, shelter, clothing, affection, education) and failure by the caregiver to ensure a child's safety within and outside the home given the child's emotional and developmental needs
Sexual maltreatment	Any completed or attempted sexual act, sexual contact with, or exploitation of a child by a caregiver; noncontact sexual maltreatment can include acts which expose a child to sexual activity (e.g., pornography), filming of a child in a sexual manner, sexual harassment, or prostitution of a child
Munchausen syndrome by proxy	Caregiver reporting nonexistent symptoms of illness in a child or deliberately causing illness in a child

Table 2
Counts of lymphocyte-subsets in maltreated children and controls

Variables	Controls	Maltreated	Test	
	(n = 19; cells/nl)	(n = 27; cells/nl)	p	d
Total lymphocytes	2.86 ± 0.77	3.09 ± 0.84	0.36	0.28
CD3 + /CD45 + total T cells	2.06 ± 0.56	2.13 ± 0.61	0.70	0.12
CD3 + CD4 + /CD45 T helper cells	1.21 ± 0.38	1.25 ± 0.38	0.70	0.12
CD31 + CD45RA + /CD4 recent thymic emigrants*	0.62 ± 0.25	0.60 ± 0.25	0.85	−0.06
CD3 + CD8 + /CD45 cytotoxic T cells	0.70 ± 0.24	0.74 ± 0.28	0.63	0.15
CD4 + HLA-DR + /CD3 activated helper T cells*	0.08 ± 0.03	0.11 ± 0.04	< 0.05	0.81
CD8 + HLA-DR + /CD3 activated cytotoxic T cells*	0.08 ± 0.04	0.14 ± 0.08	< 0.01	0.85
CD19 + /CD45 B-cells	0.56 ± 0.26	0.63 ± 0.32	0.41	0.26
CD3-CD16 + 56 + /CD45 natural killer cells	0.22 ± 0.12	0.29 ± 0.17	0.13	0.48
CD14 + /CD45 monocytes*	0.45 ± 0.10	0.49 ± 0.18	0.47	0.22
	/cell-subset in %	/cell-subset in %		
Activated helper T cells*	6.55 ± 2.25	8.97 ± 3.53	< 0.05	0.82
Activated cytotoxic T cells*	11.32 ± 4.91	18.00 ± 9.24	< 0.01	0.90

Significant p-values, large d-effect-sizes and their correspondent group means are displayed in bold.

* Maltreated: n = 24.

Table 3
Counts of lymphocyte-subsets in maltreated female children and controls

Variables	Controls	Maltreated	Test	
	(n = 11; cells/nl)	(n = 16; cells/nl)	p	d
Total lymphocytes	2.77 ± 0.82	3.26 ± 0.83	0.14	0.59
CD3 + /CD45 + total T cells	2.01 ± 0.58	2.28 ± 0.58	0.24	0.47
CD3 + CD4 + /CD45 + T helper cells	1.19 ± 0.37	1.39 ± 0.35	0.15	0.58
CD31 + CD45RA + /CD4 + recent thymic emigrants*	0.61 ± 0.28	0.74 ± 0.22	0.22	0.51
CD3 + CD8 + /CD45 + cytotoxic T cells	0.70 ± 0.28	0.74 ± 0.28	0.70	0.40
CD4 + HLA-DR + /CD + 3 activated helper T cells*	0.09 ± 0.04	0.12 ± 0.04	0.10	0.71
CD8 + HLA-DR + /CD3 + activated cytotoxic T cells*	0.07 ± 0.04	0.13 ± 0.07	< 0.05	0.99
CD19 + /CD45 + B-cells	0.52 ± 0.27	0.65 ± 0.26	0.21	0.50
CD3-CD16 + 56 + /CD45 + natural killer cells	0.22 ± 0.11	0.30 ± 0.21	0.27	0.46
CD14 + /CD45 + monocytes*	0.47 ± 0.12	0.44 ± 0.14	0.54	−0.26
	/cell-subset in %	/cell-subset in %		
Activated helper T cells*	7.34 ± 2.05	8.09 ± 2.60	0.44	0.32
Activated cytotoxic T cells*	9.90 ± 3.33	15.83 ± 6.31	< 0.01	1.18

Significant p-values, large d-effect-sizes and their correspondent group means are displayed in bold.

* Missing data for maltreated: n = 14.

Table 4
Counts of lymphocyte-subsets in maltreated male children and controls

Variables	Controls	Maltreated	Test	
	(n = 8; cells/nl)	(n = 11; cells/nl)	p	d
Total lymphocytes	2.98 ± 0.74	2.83 ± 0.82	0.69	−0.19
CD3 + /CD45 + total T cells	2.13 ± 0.57	1.91 ± 0.63	0.45	−0.36
CD3 + CD4 + /CD4 + 5 T helper cells	1.24 ± 0.41	1.05 ± 0.33	0.27	−0.52
CD31 + CD45RA + /CD4 + recent thymic emigrants	0.63 ± 0.23	0.42 ± 0.13	< 0.05	− 1.17
CD3 + CD8 + /CD45 + cytotoxic T cells	0.70 ± 0.19	0.73 ± 0.29	0.80	0.12
CD4 + HLA-DR + /CD3 + activated helper T cells	0.06 ± 0.02	0.10 ± 0.04	< 0.05	1.03
CD8 + HLA-DR + /CD3 + activated cytotoxic T cells	0.09 ± 0.04	0.15 ± 0.10	0.17	0.72
CD19 + /CD45 + B-cells	0.60 ± 0.25	0.59 ± 0.40	0.96	−0.02
CD3-CD16 + 56 + /CD45 + natural killer cells	0.23 ± 0.14	0.29 ± 0.11	0.28	0.51
CD14 + /CD45 + monocytes	0.44 ± 0.06	0.56 ± 0.21	0.11	0.79
	/cell-subset in %	/cell-subset in %		
Activated helper T cells	5.47 ± 2.17	10.21 ± 4.38	< 0.05	1.37
Activated cytotoxic T cells	13.28 ± 6.21	21.04 ± 11.97	0.12	0.81

Significant p-values, large d-effect-sizes and their correspondent group means are displayed in bold.

*Missing data for maltreated: n = 10.

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