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The Associations between Psychosocial Stress and the Frequency of Illness, and Innate and Adaptive Immune Function in Children

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Abstract

Objective—Family processes have a substantial impact on children's social and emotional well-being, but little is known about the effects of family stress on children's physical health. To begin to identify potential links between family stress and health in children, we examined associations between specific aspects of family psychosocial stress and the frequency of illnesses in children, measures of innate and adaptive immune function, and human herpesvirus 6 (HHV-6) reactivation.

Study Design—Prospective study of 169 ambulatory school-age children and parents. Parents completed multiple assessments of stress at 7 sequential six-month visits and maintained weekly illness diaries for their children over three years using a thermometer to record fever. Children had blood obtained for HHV-6 and immune function studies at each visit including natural killer (NK) cell function and the percentage of CD4 and CD8 cells associated with immune control of cytomegalovirus (CMV).

Results—Parental psychiatric symptoms were associated with a higher frequency of illnesses: for each 1 unit increase in symptom score children had an increased 1-year rate of total illnesses of 40% (rate ratio, 1.40; 95% CI, 1.06–1.85) and febrile illnesses of 77% (rate ratio, 1.77, 95% CI, 1.00–3.13). Parental psychiatric symptom scores were also associated with enhanced NK cell function (estimate, 0.15; 95% CI, 0.05–0.26) and increased percentages of CD8+CD28-CD57+ cells in the

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blood of CMV seropositive children (estimate, 2.57; 95% CI, 0.36–4.79). HHV-6 reactivation was not detected.

Conclusions—There is an association between specific psychosocial stress exposure and rates of illness and immune function in normally developing children.

Psychosocial stress adversely affects physical health. This connection has been demonstrated most convincingly in studies with diverse populations of adults, including an association between stress and an increased risk of mortality (Schulz and Beach 1999; Ickovics, Hamburger et al. 2001). Psychosocial stress also has reproducible effects on the human immune system. Chronic stress experiences of adults are associated with decreases in natural killer (NK) cell cytotoxicity, lower antibody titers to influenza vaccine, diminished delayed type hypersensitivity responses, and decreased lymphocyte proliferation (Segerstrom and Miller 2004; Glaser and Kiecolt-Glaser 2005). An association between stress and reactivation of latent herpesviruses has also been demonstrated in adult populations suggesting that psychosocial factors functionally impact effective anti-viral immunity (Glaser and Kiecolt-Glaser 2005). Evidence of decreases in both innate and antigen specific immune functions suggest that as stress becomes more chronic, an increasing number of components of the immune system are affected in potentially adverse ways (Segerstrom and Miller 2004). Despite these findings, however, changes in immune function due to psychosocial stress are not reliably associated with disease susceptibility.

How findings from reports of stress and adult diseases apply to children remains to be determined. Although family stress is reliably associated with increases in children's risk for maladaptive social, emotional, and cognitive development (Conger, Conger et al. 1992; Masten, Hubbard et al. 1999; Costello, Compton et al. 2003), only limited research has examined how children's experiences of stress influences their health and immune function. There is a suggestion that stress may be associated with streptococcal pharyngitis and upper respiratory illnesses in children (Meyer 1962; Graham 1986; Clover 1989; Cobb and Steptoe 1998). However, few such studies have included measures of specific psychosocial stressors and health outcomes, or examined these links longitudinally.

The effect of psychosocial stress on the immune function of children, in particular, is under–explored. Preliminary reports suggest that the effects of stress on children's immunity may differ from adults. One recent report described an association in infants between parenting stress, parental unemployment, serious adverse events during the first year of life and the presence of high concentrations of autoantibodies associated with type-one diabetes (Sepa, Wahlberg et al. 2005). Among young children predisposed to asthma, high levels of parent reported stress predicted greater atopic immune profiles, including increases in proliferative responses (Wright, Finn et al. 2004). Similarly, Chen and colleagues reported that high levels of stress among adolescents with histories of asthma predicted lower morning cortisol levels and higher stimulated cytokine responses indicative of elevations in both Th-2 and Th-1 responses (Chen, Fisher et al. 2003). One of the hypothesized mechanisms by which psychosocial stress effects immune function and health is via activation of the hypothalamic-pituitary-adrenal (HPA) axis with dysregulation of cortisol secretion (Glaser and Kiecolt-Glaser 2005; Flinn 2006). The findings of Chen and colleagues are consistent with data demonstrating that chronic stress may decrease cortisol responses in children (Flinn 2006). The immunological data provided by the studies outlined above are equally important, but have no ready interpretation, and they contrast – at least in principle – with the adult-based model that presumes that stress dampens immune function.

To expand on existing work linking stress and child health, we began a prospective, longitudinal study of a diverse cohort of school-age children. Our hypotheses were that elevated levels of specific psychosocial stresses would be associated with an increased number of

illnesses and febrile illnesses in children, changes in specific immune system parameters involved in the control of viral replication, and increased rates of reactivation of human herpesvirus 6 (HHV-6). We focused on psychosocial stress in children's lives that are widely researched and repeatedly linked with children's behavioral adjustment; namely: parental psychiatric symptoms and functioning, stressful life events, and family conflict (Sameroff 1983). We hypothesized that these sources of stress may also predict physical health. In choosing immunological outcome variables, we considered both innate and antigen specific aspects of immune function involved in the control of common, persistent, viral infections. These included NK cell function as a measure of innate immune function, as changes are noted in these cells during chronic viral infections (Biron, Nguyen et al. 1999). There is also a well documented association between prior cytomegalovirus (CMV) infection and marked oligoclonal expansion of the CD8+CD57+ subset of T cells, which have of late been shown to be terminally differentiated CD8+ T cells that lack either the CD27 or CD28 surface marker (Wang, Taylor-Wiedeman et al. 1993; Wang and Borysiewicz 1995; Batliwalla, Monteiro et al. 1996; Hooper, Kallas et al. 1999; Kuijpers, Vossen et al. 2003). We chose to measure the frequency of these cells as a marker of adaptive immune function, and hypothesized that the percentage of CD8+CD28-CD57+ cells would be increased in children with prior CMV infection living in environments with the highest degree of stress, reflecting episodic/sporadic viral antigen stimulation with the occurrence of CMV reactivation. As an indirect measure of antigen specific immune function, we also examined HHV-6 activity, as this virus is acquired by all children by three years of age and has been shown to reactivate and cause recurrent disease in immunocompromised hosts (Caserta, Mock et al. 2001).

A subset of results of the first 18 months of this study have been previously reported and demonstrated that children living with parents reporting increased levels of psychiatric symptoms in the context of family stress had more total illnesses and febrile illnesses over a one year period compared to children in less stressed families (Wyman PA 2007). In addition, children living in families with higher stress had enhanced NK cell function over an 18-month period. This report extends these findings by defining more precisely the psychosocial factors associated with child health over the full three-year period of study and by examining child temperament as a moderating factor of family-stress illness relationships. The innate immune function outcome (NK cell function) that was measured in the children over the entire course of the study is now presented. In addition, this study extends outcomes to include an adaptive immunity measure not included in our prior report.

METHODS

Subject Enrollment and Study Protocol

Children between 5–10 years of age and one of their primary caregivers were recruited between July 1, 2001 and June 30, 2003 from an ambulatory population already participating in a study of pediatric viral infections at the University of Rochester School of Medicine. The children were initially identified by visits to the emergency department or other pediatric services. Children with chronic diseases affecting the immune system (e.g., receiving chronic corticosteroid therapy) were excluded. One child per family was enrolled and all children were well at the first visit. More than 90% of the enrolled caregivers were children's biological or adoptive parents; therefore, the term parent is used throughout this manuscript. The RSRB of the University of Rochester approved this study and all parents provided informed consent. A $45 honorarium was provided to the family following each visit.

The protocol consisted of 7 visits, approximately 6 months apart over three years. At each visit parents completed measures of stress in the parent and family unit, provided an interim medical history and reported any symptoms of illness in their child in the preceding two weeks. Vital

signs, height, and weight of the children were measured at each visit and blood samples were collected.

Psychosocial Stress Measures

Psychosocial stress measures completed by parents were as follows: (1) Parent psychiatric symptoms were ascertained at each visit (6-month intervals) using the 51-item Brief Symptom Inventory (BSI) (Derogatis 1992). BSI items are scored on a severity rating of 0 to 4 based on the prior month. The items assess nine symptom clusters including depression, anxiety, and psychoticism. The total symptom score (item average, GSI or Global Symptom Index) was used, which has been shown to be sensitive to changes in psychological status arising from mental disorders and social-interpersonal events. For the BSI, clinically significant threshold values on the total symptom scores are defined separately for women and men and correspond to the highest 10% in typical non-clinical samples of adults (Derogatis 1992). (2) Parents completed the Stressful Life Events and Conditions Checklist (SLECC) (Kilmer 1998) at study entry, and reported which of 35 adverse stress events and chronic processes had occurred since the child's birth. The SLECC items cover five empirically identified stress factors: family turmoil, family separation, poverty, neighborhood violence, and family illness/injury. At each of the subsequent visits, parents reported which stressors were ongoing or had newly occurred, and rated the severity of each on a numerical scale (0–4). This measure is scored as the total number of events with a range of 0–35. (3) An 8-item family conflict measure was completed at every visit with a range of 8–32 on the final score (Black and Pedro-Carroll 1993). Items assess frequency and intensity of conflict between family members and resolution. (4)The Parent Isolation and Attachment Problem subscales from the Parenting Stress Inventory (PSI) (Abidin 1995) assessed parent-child relationship stress at enrollment and again one year later. These subscales assess parental report of interpersonal distance due to parent role and lack of satisfaction with the child-parent relationship (5) Adult-Adolescent Parenting Inventory (AAPI) (Bavolek 1984) captured attitudes associated with child maltreatment and was completed at the second visit and then one year later. The AAPI items load on four empirically-derived factors: inappropriate developmental expectations, lack of empathy for child, role reversal, and physical punishment used in parenting. Higher scores on the BSI, SLECC, family conflict measure, and PSI indicate more stress and dissatisfaction with parent role.

We also included a child factor that we hypothesized would moderate the links between stress and health: children's negative affectivity, rated on a standardized measure of temperament. Parents completed the Children's Behavior Questionnaire (Rothbart 2001) at baseline and one year later for children younger than 7.5 years. For children older than 7.5 years, parents completed at baseline and one year later the corresponding Early Adolescent Temperament Questionnaire (Capaldi 1992). The two ratings of each child, completed one year apart, were averaged to form a negative affectivity score.

Illness Diary

Parents were instructed to record their child's health once weekly on a prepared form and were provided a digital thermometer. Definitions of illness were not provided; rather, parents were asked to record all episodes they considered illnesses with the corresponding temperature and symptoms. Diaries were returned monthly. If not returned, study nurses contacted the family by phone. If the parent could not be reached, the diary was updated at the next visit. Illness reports related to trauma, elective or orthopedic surgery, mental health/behavioral problems, and constipation were excluded. Fever was defined as a temperature >38°C. If a parent reported fever, but a temperature was not provided, the illness was recorded as a febrile illness. If families reported a fever, but the recorded temperature was ≤ 38°C, the illness was recoded as without fever.

Laboratory Assays

CMV serology was performed at study entry by the clinical microbiology laboratory utilizing the Bio-Merieux Vidas instrument and CMV IgG kit (Bio-Merieux SA., Marci L'Etoile, France).

A fluorescent activated cell sorting (FACS) assay was performed for immune cell phenotyping as previously described (Jin 2002). Lymphocytes were gated on the basis of forward and side scatter; a minimum of 20,000 cells were collected for analysis for each sample. Data analysis was performed using CellQuest™ software (Becton-Dickinson, San Jose, CA). The immunophenotyping panel was: CD3/CD16/CD56 for NK cells, CD4/CD28/CD57, and CD8/CD28/CD57. All monoclonal antibodies were purchased from BD PharMingen (San Diego, CA) and conjugated with either fluorescein isothiocyanate, phycoerythrin, or Cy-Chrome.

NK cell cytotoxicity assays were performed on whole blood samples as previously reported (Bromelow, Galea-Lauri et al. 1998). The percent specific lysis was calculated as: 100x(counts per minute (cpm) experimental-cpm spontaneous)/(cpm maximum-cpm spontaneous) for each dilution of whole blood. The percent specific lysis at each dilution was log transformed, and linear regression was used to estimate the dilution that corresponded to 20% lysis, which was then used as a measure of NK cell cytotoxicity for each visit.

HHV-6 qualitative polymerase chain reaction (PCR) and reverse transcriptase-polymerase chain reaction (RT-PCR) assays were performed as previously described (Hall, Long et al. 1994; Norton, Caserta et al. 1999). Samples that produced a positive result on qualitative testing had a viral load measurement. Real-time quantitative PCR for the U38 gene of HHV-6 was performed as described (Zhen, Bradel-Tretheway et al. 2005; Caserta 2007)

Statistical Analysis

The General Estimating Equation method (GEE) (Zeger and Liang 1986), with a log link, was used to test the association between chronic stress and parent-reported total illnesses and febrile illnesses over the three year study. The GEE methodology accommodates correlated and missing data and provides robust estimates. Our initial report from the first 18 months of this study utilized factor analyses in order to maximize statistical power from a limited period of follow-up, and identified BSI total, SLECC total and family conflict as combining into a parent distress factor that accounted for a large proportion of variance in parent measures. Our goal in the present report was to determine the contribution of each of these individual measures of stress in the family to child health and immune function over the full three years of study. Also, we aimed to focus on those three specific measures of psychosocial stress which have all been widely researched and repeatedly linked with children's behavioral adjustment: parental psychiatric symptoms, stressful life events, and family conflict. In order to analyze all three years of illness data the numbers of parent-reported illnesses and febrile illnesses in the one year following visits 1, 3, and 5 were the dependent variables and the stress measures (BSI, SLECC, and family conflict) administered at the visit beginning each 1-year period were independent variables, thus, reducing seasonal effects on children's illnesses. Child age, sex, race and annual household income were included as additional covariates in the model.

To examine the association between chronic stress and immune function, longitudinal data analysis was performed for all seven visits using mixed models with random subject effects to account for within-subject correlation over time (Laird 1982). The outcome variable was NK cell function, and the stress measures obtained prior to each assessment of immune function were used as time-varying predictors. Child age, sex, race, and annual household income were included in the model. NK cell function was log-transformed for normality. To determine if illness close to or at the time of the visit would significantly alter the effect on NK cell function,

the analysis was repeated including an extra indicator variable for any report of illness for the child within the preceding 14 days. This model was used to estimate the mean change in the natural logarithm of NK cell function with 1-unit change in each stress score. Similar analyses were repeated with percentage of CD8+CD28-CD57+ cells, and CD4+CD28-CD57+ cells in CMV seropositive children, and the HHV-6 viral load at each visit as outcome variables respectively.

Mediation analysis (Baron 1986) was performed to test if NK cell function and the percentage of CD8+CD28-CD57+ cells mediate the relationship between family stress and child illnesses. The GEE model procedures with a log link were repeated to test the moderation effect of parent report of child negative affectivity on family stress predicting child illnesses.

Analyses were performed with SAS (version 9.1, SAS Institute Inc, Cary, NC).

RESULTS

Cohort Description

We enrolled 170 racially and socio-economically diverse, ambulatory children and one of their parents into this study (Table 1). Data for one child were removed from the analyses because he did not meet inclusion criteria, leaving 169 children. The parents' mean age at enrollment was 35 years (range 21–73 years), 93% (158) were female and 151 were mothers of participants. At visit four, 149 (88%) subjects remained and 120 (71%) completed all seven visits. Reasons for leaving the study included non-compliance with follow-up visits (30), relocation out of area (7), lack of interest (12), and anxiety around school entry (1).

We compared the distributions of child age, annual household income, and stress measures at visit 1 between subjects who completed 7 visits and those who did not and found no significant differences. We further tested the statistical assumption of Missing Completely at Random (MCAR). This analysis demonstrated that none of the previously observed demographic or stress measures were significantly associated with the probability of patient withdrawal. Thus the data have a monotone missing pattern (Robins 1995) and our assumption of MCAR is valid. Based upon these analyses one can conclude that the missing data did not significantly alter the results. In a longitudinal study with MCAR data, the GEE and the linear mixed model provide consistent estimators of regression coefficients (Diggle 2002). These analyses incorporate all of the data that is present instead of listwise deleting subjects with missing values

The total number of illnesses recorded during the study was 1065. Among the 330 reported febrile illnesses, 230 had temperatures recorded. The mean temperature of febrile illnesses was 38.9°C. Parents reported more illnesses in their children during the winter months of October 16-April15 (59 to 77% of all illnesses recorded) than during the spring and summer, consistent with seasonal trends.

Child Illnesses and Febrile Illness

Parent reported psychiatric symptoms, as measured by BSI, were associated with child illnesses over 3 years (Table 2). For each 1-unit increase in parental BSI score children had an increased rate of total illnesses of 40% (rate ratio, 1.40; 95% CI, 1.06–1.85) (Table 2) and a 77% (rate ratio, 1.77, 95% CI, 1.00–3.13) increased rate of febrile illnesses. Effect sizes were slightly lower (but still significant) when we examined BSI according to clinical cut-off scores. For children of parents with BSI scores in the clinically significant range, compared to parents in the normal range, the risk ratio of having at least one reported illness over the following year was 1.21 (95%CI, 1.06 –1.39) after adjusting for child age, sex, race and household income. The adjusted risk ratio of having at least one reported febrile illness over the following year

was 1.37 (95% CI, 1.05 – 1.77). Neither stressful life events (SLECC) nor family conflict were significantly associated with child illnesses or febrile illnesses. Younger children did have significantly more illnesses and febrile illnesses than older children, consistent with previous reports.

Analyses to test moderation of stress on febrile and non-febrile illnesses

GEE analysis was repeated with the model including both main effects and interaction effects. Results indicated that the association between BSI and illness and febrile illness was not significantly moderated by parent rating of children's negative affectivity, child age, race, or gender.

Children's Innate and Adaptive Immune Responses

We performed 1002 NK cell assays during the study; 69 (7%) were excluded due to insufficient data. The intraclass correlation coefficient of NK cell function was 0.32. Median NK cell function had an overall range of 0.4 to 44.18.

Over three years of study, children with parents having higher BSI scores were found to have enhanced NK cell function. After controlling for age, sex, race, and household income, for each 1-unit increase in BSI score, the natural log of NK cell function increased by 0.15 units (95% CI, 0.05–0.26) (Table 3). Family conflict was negatively associated with NK cell function. Each 1-unit increase in the family conflict score was associated with 0.01 unit decrease in natural log of NK cell function (95% CI, −0.03 – −0.00). The correlation between BSI and family conflict was 0.41. We repeated the analyses controlling for any illness in the child in the 14 days preceding the visit because recent illness might influence NK cell function. The associations between BSI and NK cell function and family conflict and NK cell function remained significant. Occurrence of illness in the two weeks before the study visit was associated with 0.08 unit increase in natural log of NK cell function (95% CI, 0.02 – 0.14), consistent with a recent activation due to infection.

We measured the percentage of CD8+CD28-CD57+ cells in the peripheral blood by FACS analysis on 995 samples. This cell population has been used as a surrogate for CMV-specific T cell activity (Hooper, Kallas et al. 1999; Kuijpers, Vossen et al. 2003). The mean percentage of CD8+CD28-CD57+ cells measured was significantly greater in CMV seropositive children (7.8%) than CMV seronegative children (3.3%) (mean difference, 4.5%; 95% CI, 3.8 – 5.2), confirming the association between this cell population and prior CMV infection. As a secondary analysis we also re-analyzed a subset of FACS results from 23 of 40 CMV seropositive children from visit 6 based upon the availability of sufficient quality FACS data. A significant majority ($p<.0001$) of the CD8+CD28-CD57+ cells were in the $CD8^{hi}$ subset indicating that most of these cells are terminally differentiated T cells and not NK cells (details available from the first author).

In the 40 CMV seropositive children, for each 1-unit increase in BSI score, the mean percentage of CD8+CD28-CD57+ cells measured in the blood increased by 2.57 percent (95% CI, 0.36–4.79) (Table 4). This association was not significant in CMV seronegative children (p-value=0.26). Also, there was no significant association between any psychosocial measure and the percentage of CD4+CD28-CD57+ cells in CMV seropositive children.

HHV-6 Reactivation

HHV-6 DNA was identified in 468 of 1011 peripheral blood mononuclear cell samples. The RT-PCR assay identified only one episode of reactivation over the 5 years of study. Thus, this outcome was identified too infrequently to be meaningful. There was no significant association

identified between any stress measure and the likelihood of identifying HHV-6 DNA in the blood, or with the viral load of HHV-6 DNA detected.

Analyses to test moderation of stress on immune parameters and mediation of illnesses

GEE analysis was again used to test for moderation. Results indicated that the significant associations between psychosocial stress measures and specific immune outcomes were not moderated by parent reports of children's negative affectivity, age, gender, or race. Further analyses indicated that neither NK cell function nor the percentage of CD8+CD28-CD57+ cells in the peripheral blood of children mediated the effect of psychosocial stress on illnesses or febrile illnesses; that is, we found no evidence of mediation.

DISCUSSION

Findings from the current study extend the literature on psychosocial stress and illness in children in three important ways. First, the frequency of illnesses and febrile illnesses in a diverse sample of generally healthy children observed over a three-year period was significantly associated with parent's self-reported psychiatric symptoms. Although previous studies have found an association between measures of psychosocial stress in families and various types of child illnesses, many are limited by the use of a cross-sectional design, retrospective collection of illness data, global reports of child health, or short term follow-up (Graham, Woodward et al. 1990; Cobb and Steptoe 1998; Graham-Bermann and Seng 2005). Findings from the present study are robust because they derive from a prospective design coupled with multiple occasions of measurement over a 3-year follow-up; furthermore, we recorded illnesses using an objective index, namely, the measurement of fever. Both of these study features are improvements on existing research and add to the validity of the findings.

Second, we sought to clarify the nature of stress exposure that is linked with compromised health in children. Meyer and Haggerty found an association between family stress and streptococcal infections and illnesses, but were unable to determine the mechanism of the connection (Meyer 1962). More recent reports that rely on composited indices of stress may be difficult to translate to clinical intervention and prevention (Graham, Woodward et al. 1990; Klinnert, Nelson et al. 2001). We limited our focus to stresses that have been extensively studied in psychosocial research, and to a largely normative, predominantly lower income sample without underlying illness predispositions; furthermore, we sought to identify specific sources of stress. Parental psychiatric symptoms were significantly associated with child illnesses, febrile illnesses, and measures of immune function. These findings extend prior animal research (Newport, Stowe et al. 2002) and a more recent cross-sectional study that found that children with asthma had mothers with more self-reported depressive symptoms (Shalowitz, Mijanovich et al. 2006). Further research exploring the components of maternal mood and psychiatric symptoms that impair children's health, and the mechanisms involved in these interactions, may lead to potential intervention strategies with broad public health implications.

Third, our research design provided considerable leverage for understanding the biological impact of multiple sources of chronic stress in families on specific immune functions in a sample of generally healthy children. In prior studies of children and adolescents with genetic predisposition to asthma or ongoing asthma symptoms, psychosocial stress measures have been associated with serum levels of IgE, proliferative responses to mitogens, and mitogen stimulated cytokine responses (Chen, Fisher et al. 2003; Wright, Finn et al. 2004). However, there is a paucity of data on antigen specific immune responses and psychosocial stress. Thus, our findings that parental psychiatric symptoms were associated with an increased percentage of CD8+CD28-CD57+ cells in the blood of children with prior CMV infection is novel. This effector memory cell population has been shown to be significantly associated with the long-

term immunological control of CMV, and not other common viruses (Kuijpers, Vossen et al. 2003). A higher percentage of CD8+CD28-CD57+ cells has been identified in immunosuppressed renal transplant patients following primary CMV infection compared to normal CMV seropositive controls, indicating that the frequency of these cells is directly associated with increased CMV activity (Kuijpers, Vossen et al. 2003). Our results suggest that children of parents with more psychiatric symptoms have impaired immune control of CMV replication, implying diminished antigen specific responses that have not yet been identified. We did not find an association between any psychosocial variables and reactivation of HHV-6. Unfortunately, our protocol did not allow for more frequent specimen collections, and, therefore, we did not detect reactivation with the RT-PCR assay for HHV-6 in a sufficient number of subjects for meaningful analysis.

Parents with elevated psychiatric symptoms as measured by BSI (largely attributable to anxiety and depression) also had children with enhanced NK cell function. These data are consistent with our findings from the first 18 months of the study and extend these findings from the first 18 months of study to 3 full years. Although this finding is contrary to what has been reported in older adults, it is consistent with a small study of adolescents that found enhanced NK cell function in depressed subjects compared to controls (Schleifer, Bartlett et al. 2002). As noted previously, contrary findings exist in the literature of stress and immune function between adults and children. The observation that the same measure of parental psychiatric symptoms was associated with more illnesses and alterations in adaptive immune responses, suggesting poor immune control of CMV, implies that enhanced NK cell function may have different implications than previously suggested.

Of the remaining stress measures evaluated, family conflict emerged as significant and was associated with diminished NK cell function. Why parental psychiatric symptoms were more often significantly associated with immune function measures compared to the other indicators is not yet clear and requires further investigation. Parental psychiatric symptoms represent a broad risk, both in terms of potential mechanisms of action (e.g., genetic as well as environmental) and developmental processes (e.g., quality of parental care, exposure to affiliated risks which are disproportional for symptomatic individuals). It may be that such a broad index is needed to detect a reliable signal connecting risk exposure and illness.

Limitations to the study include parental reports of illness and fever without clinical corroboration in one-third of febrile illnesses recorded by parents. However, prior research has demonstrated the validity of parental reports of febrile illnesses when a thermometer reading is provided, which occurred in the majority of family reports of fever (Bonadio, Hegenbarth et al. 1990). In addition, we did not ask the parents to obtain temperature readings from their children when an illness was not suspected which may have decreased the sensitivity of parent report, especially from less attentive parents. However, requesting frequent temperature monitoring from the families was both not feasible and had the potential to induce undue parental concern.

Additionally, the association of parental BSI symptoms with the frequency of CD8+CD28-CD57+ cells as a marker of adaptive immunity may have been confounded by the addition of a minor population of $CD8^{lo}$ cells (NK cells) in the assay. However, our secondary analysis demonstrated that a significant majority of these cells were $CD8^{hi}$ consistent with effector T cells. Also there was no association between the CD8+CD28-CD57+ subset of cells and BSI scores in CMV seronegative children implying that the change in frequency observed in this cell population was related to prior infection with CMV, and not merely due to the increased frequency of NK cells associated with parental BSI scores detected in the whole group of children.

A separate limitation was the failure to detect moderation in our exploratory analyses, which may have been due to small sample sizes and small effect sizes. These limitations are offset by several strengths, including the prospective longitudinal design, multiple occasions of measurement (7), inclusion of multiple, coherent indicators of stress exposure and immune function in children.

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Table 1

Subject Characteristics at Enrollment

	Subject Characteristics (n=169)	Baseline BSI* Mean (SD)	At Baseline, Parents with Clinically Significant BSI n (%)	Baseline Family Conflict^ Mean (SD)	Baseline SLECC+ Mean (SD)
Child's Race:					
White	78 (46%)	0.49 (0.38)	21(27%)	14.45 (3.87)	5.72 (4.15)
Black	49 (29%)	0.57 (0.46)	12 (24%)	14.67 (3.66)	6.57 (3.87)
Hispanic	11 (7%)	0.94 (0.50)	7 (64%)	14.73 (3.52)	4.45 (3.24)
Asian	4 (2%)	0.33 (0.29)	0 (0%)	11.50 (4.43)	0.75 (0.96)
Biracial	27 (16%)	0.60 (0.45)	8 (30%)	15.48 (4.89)	7.74 (4.23)
Child's Gender:					
Male	87 (51%)	0.59 (0.42)	25 (29%)	15.16 (3.94)	6.43 (4.34)
Female	82 (49%)	0.53 (0.42)	23 (28%)	14.06 (3.98)	5.73 (3.87)
Child's Age:					
5 – 6 Years	78 (46%)	0.63 (0.48)	28 (36%)	14.09 (4.24)	5.41 (3.82)
7 – 10 Years	91 (54%)	0.50 (0.38)	20 (22%)	15.09 (3.71)	6.67 (4.30)
Household Income:					
<= $25K	59 (35%)	0.64 (0.46)	21 (36%)	14.27 (3.92)	6.54 (3.56)
$26K-55K	63 (38%)	0.58 (0.44)	19 (30%)	16.00 (4.04)	6.95 (4.75)
$56K-95K	31 (19%)	0.44 (0.38)	5 (16%)	13.03 (3.34)	4.20 (3.11)
$95K<	14 (8%)	0.42 (0.32)	3 (21%)	14.00 (3.82)	3.93 (2.95)

*BSI, Brief Symptom Inventory, sum score is item average (range 0 – 4).

^Family Conflict scores are sum of 8 items on 4-point scale (range 8 – 32).

+SLECC, Stressful Life Events and Conditions Checklist, scored as number of events (range 0–35). For this cohort, the ranges at baseline are BSI (0 – 2.18), Family Conflict (8 – 27), SLECC (0 – 21).

Table 2

Estimated Rate Ratios for the Association between Stress Measures and Total Illness and Febrile Illnesses in Children.

Variables	All illnesses RR (95% CI)	P Value	Febrile illnesses RR (95% CI)	P Value
BSI	1.40 (1.06, 1.85)	0.02	1.77 (1.00, 3.13)	0.05
SLECC at entry	1.00 (0.96, 1.04)	0.98	0.97 (0.85, 1.11)	0.68
SLECC	1.00 (0.98, 1.02)	0.99	1.01 (0.95, 1.08)	0.69
Family conflict	0.99 (0.96, 1.03)	0.70	1.01 (0.93, 1.10)	0.75

Abbreviations: BSI, Brief Symptom Inventory; CI, confidence interval; RR, rate ratio; SLECC, Stressful Life Events and Conditions Checklist.

* Analyses were adjusted for child age, sex, race and annual household income.

For each variable, the estimated rate ratio is for a 1-U increase.

Data from169 subjects were included; 22 of them had some visits with missing values.

Table 3

Adjusted Mean Differences in the Natural Log of Natural Killer (NK) Cell function for Study Variables*

Variable	NK Cell Function Estimate (95% CI)	P Value
BSI	0.15 (0.05, 0.26)	< 0.01
SLECC	0.00 (−0.01, 0.01)	0.81
Family conflict	−0.01 (−0.03, 0.00)	< 0.01
Sick prior 14 Days	0.08 (0.02, 0.14)	0.01

Abbreviations: BSI, Brief Symptom Inventory; CI, confidence interval; SLECC, Stressful Life Events and Conditions Checklist.

*Analyses were adjusted for child age, sex, race, and annual household income. For each variable, the estimated difference is for a 1-U increase.

Data from 169 subjects were included; 52 subjects had some visits with missing values.

Table 4

Adjusted Mean Differences in the Percentage of CD8+CD28-CD57+ Cells in the Peripheral Blood of CMV Seropositive Children*

Variable	Percentage of CD8+CD28-CD57+ Cells	
	Estimate (95% CI)	P Value
BSI	2.57 (0.36, 4.79)	0.02
SLECC	−0.06 (−0.19, 0.08)	0.41
Family conflict	0.01 (−0.24, 0.25)	0.97

Abbreviations: BSI, Brief Symptom Inventory; CI, confidence interval; SLECC, Stressful Life Events and Conditions Checklist.

* Analyses were adjusted for child age, sex, race, and annual household income. For each variable, the estimated difference is for a 1-U increase.

Data from 39 subjects were included in the analysis, 12 of them had some visits with missing values.