Association of Family Stress With Natural Killer Cell Activity and the Frequency of Illnesses in Children

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Objective: To examine prospective associations between chronic stress in the parent-child and family systems and subsequent rates of illnesses and the activity of natural killer (NK) cells in children.

Design: Prospective cohort study.


Participants: One hundred sixty-nine socioeconomically and racially diverse children (aged 5-10 years) and their parents. Parents completed measures of their psychiatric symptoms and stress in the family every 6 months. Children’s blood samples were obtained for NK cytotoxicity assays every 6 months.

Main Outcome Measures: Parent-reported total child illnesses and febrile illnesses and results of NK cell cytotoxicity assays. We estimated adjusted mean differences in NK activity.

Results: Elevated parental psychiatric symptoms occurring with family stressors were associated with more total illnesses (rate ratio, 1.11; 95% confidence interval [CI], 1.00-1.22) and febrile illnesses (rate ratio, 1.36; 95% CI, 1.13-1.64) in children. Natural killer cell function was enhanced in children whose parents reported more chronic stress (estimate, 0.15; 95% CI, 0.05-0.26). Natural killer cell function was not associated with short-term changes in stress. Stress-illness relationships were not associated with stress-related alterations in NK cell function.

Conclusions: Chronic family stress was associated with increased illnesses in children. Unlike older adults, children living with elevated chronic stress had enhanced rather than decreased NK cytotoxicity, suggesting chronic stress may have different effects on the developing immune system. Impaired parental functioning may be a mechanism linking family stress with adverse effects on children’s health.

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The human response to stress is linked to alterations in immune function and to several diseases in adults, including a heightened risk for cardiovascular disease and increased susceptibility to infectious diseases. In general, stress has a negative effect on health, and in certain groups stress is associated with an increased risk of mortality.

Chronic stress in adults is consistently associated with decreases in natural killer (NK) cell cytotoxicity, lower antibody titers to the influenza vaccine, and decreased lymphocyte proliferation. Mechanisms linking stress and increased susceptibility to infectious diseases with specific alterations in immune functions, however, have not been identified.

Only a modest number of studies have examined the effect of children’s experiences of stress on their health. The findings from those studies vary, and the mechanisms governing the stress-health relationship for children are largely unknown. Boyce and colleagues reported increased levels of respiratory illness in children associated with school stress, but only in those children with the highest level of reported reactivity to a short laboratory task or to a naturalistic stressor. Increased frequencies of streptococcal infections and illnesses were found among children in families with greater amounts of chronic stress. Stress scores, however, were determined by the researchers on the basis of a global score of family function without determining the active mechanisms of stress. A study of new mothers found a modest association between higher maternal stress and increased lower respiratory tract infections in their infants. Few studies have examined the effects of stress on children’s immune function. Among young children predisposed to atopy and asthma, high levels of parental stress predicted increases in proliferative responses to selected antigens, suggesting that genotype may influence the
effect of stress on children. Little is known about the influence of different stress experiences on the health and immune function of the developing child and whether chronic childhood stress alters immune function in ways that have meaningful implications for disease susceptibility.

The present study examined associations between sources of stress known to alter parent-child relationships and to adversely affect children's functioning and the rate of illnesses in children, as well as children's NK cell function.

METHODS

SUBJECT ENROLLMENT AND STUDY PROTOCOL

Children (aged 5-10 years) were recruited from a population already participating in a study of childhood infections at the University of Rochester School of Medicine and Dentistry. Participants were initially identified by visits to the emergency department or other pediatric services of the Golisano Children's Hospital at Strong, Rochester. Subjects enrolled in the new study from July 1, 2001, through June 30, 2003. Only 1 child per family was enrolled, and all were healthy at enrollment. Children with chronic conditions affecting the immune system (eg, asthma requiring long-term corticosteroid therapy) were excluded. One subject was not included in the analyses because he did not meet the inclusion criteria after enrollment. More than 93% of the enrolled caregivers were children's biological parents; therefore, we use the term parent throughout the remainder of the article. The institutional review board of the University of Rochester approved this study, and all parents provided informed consent. A $45 honorarium was provided to the family after each visit.

The protocol consisted of 7 visits, approximately 6 months apart. We report data from the first 4 visits, encompassing 18 months of follow-up. At each visit, parents completed measures of personal and family stress and provided an interim medical history for their child. Children engaged in quiet activities in a separate room. At the end of each visit, blood was obtained from each child by venipuncture after the use of anesthetic cream.

ILLNESS DIARY

At enrollment, parents were given a digital thermometer and instructed to record their child's health weekly. Strict definitions of illness were not provided, but parents were encouraged to record all episodes they considered illnesses with the corresponding temperature and symptoms. If the diaries were not returned monthly, study nurses contacted the family to collect the information. If the family could not be reached, the diary was updated at the next visit. Fever was defined as a temperature higher than 38°C. If the parents reported fever without a recorded temperature, the illness was recorded as febrile. If families reported fever but the temperature was 38°C or lower, the illness was recorded as nonebrile.

Reports of well-child care, trauma, elective or orthopedic surgery, and mental health or behavioral problems were excluded. Allergies, asthma, eczema, and contact dermatitis were included in the illness counts.

STRESS MEASURES

Parents completed the following measures of personal and family stress. (1) The 51-item Brief Symptom Inventory (BSI) was completed every 6 months to assess psychiatric symptoms, including depression and anxiety. (2) Parents used the Stressful Life Events and Conditions Checklist (SLECC) at study entry to indicate which of 35 adverse events (eg, violence exposure) and chronic processes (eg, parental unemployment) had occurred since the child's birth. At each subsequent visit, parents reported which events were ongoing or had newly occurred during the preceding 6-month interval and rated the severity of each event on a numerical scale. (3) The parental isolation and attachment problem subscales from the Parenting Stress Inventory (PSI) assessed parent-child relationship stress at enrollment and again 1 year later. (4) The Adult-Adolescent Parenting Inventory (AAPI) assessed attitudes associated with child maltreatment and was completed at visit 2 and again 1 year later. (5) An 8-item family conflict measure was completed at each visit. Higher scores on the BSI, SLECC, PSI, and family conflict measures indicate higher levels of stress, whereas higher scores on the AAPI indicated more competent parenting attitudes. For the BSI, PSI, and AAPI, clinically significant values are defined.

DIMENSIONS OF PARENT-CHILD SYSTEM STRESS

It is well established that multiple family stressors significantly increase a child's risk for maladaptation and that impaired parental functioning with children is a salient mechanism by which family stressors affect children. We therefore used factor analysis to identify a combination of measures reflecting chronic stress in the parent-child system that would not be evident in a single measure. For analysis of illnesses, the following 7 stress measures from visits 1 and 2 were entered into a factor analysis: BSI, SLECC at entry, SLECC at visit 2, PSI isolation, PSI attachment, AAPI, and family conflict. For measures that were repeated at each visit (BSI and family conflict), a mean of the 2 visit scores was used. Using the standard varimax rotation of the principal components, we identified a 2-factor solution. The first factor was most heavily weighted with elevated parental psychiatric symptoms (BSI) and elevated family stressors (SLECC) reflecting a parent impaired by family stress (factor 1), with a higher score indicative of greater stress. The second factor was most heavily weighted by negative parenting attitudes (AAPI) and parenting stress (PSI) and was designated as parenting role stress (factor 2), with a lower score indicative of more stress. For analysis of NK cell function, a second factor analysis was applied to the means of the 7 stress measures across all 4 visits, and a similar 2-factor solution was identified. To determine whether the factors were reliable, we used the standard practice of randomly dividing the sample into 2 halves and repeating the derivation of the factors in each half, confirming the same 2-factor solution.

LABORATORY METHODS

The NK cell cytotoxicity assays were performed on whole blood samples the day of the visit as previously reported. The percentage specific lysis was calculated by using the following equation for each dilution:

$$100 \times \frac{(\text{CPM Experimental} - \text{CPM Spontaneous})}{(\text{CPM Maximum} - \text{CPM Spontaneous})}$$

in which CPM indicates counts per minute. Each dilution was log transformed. Linear regression was used to estimate the dilution that corresponded to 20% lysis, which was used as an overall measure of NK cell cytotoxicity.

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STATISTICAL METHODS

If 10% or fewer of the items on a psychosocial measure had missing values, the mean of the nonmissing items was used to fill in the blanks, whereas if more than 10% of the items were missing, the score was not used.

For our primary analyses, the number of parent-reported illnesses and febrile illnesses in the 1 year following visit 2 was tallied, and adjusted rate ratios with 95% confidence intervals (CIs) were estimated using Poisson regression analysis. All illnesses reported during the 1-year period were included to avoid seasonal bias, and the Poisson models were adjusted for the actual length of the reporting time and checked for overdispersion, which was not a problem. Factors 1 (parent impaired by family stress) and 2 (parenting role stress) were included in each model. Age (as a continuous variable), sex, and race (white, black, or other) of the child and annual household income per person at visit 4 were included in the models. As a secondary analysis, visit 2 stress variables (BSI, SLECC, AAPI, and family conflict) replaced the factors in a separate model.

We additionally sought to examine the association between chronic stress and children’s immune function as manifested by NK cell function. The primary analysis used multiple (linear) regression analysis that included the factor scores based on the means of all of the psychosocial measures from the first 4 visits as independent variables to capture the effect of chronic stress during a 1½-year period. Natural killer cell function at visit 4 was the outcome variable. Child age, sex, and race and annual household income per person at visit 4 were included in the model. A secondary analysis replaced the factors with the means of each individual stress variable (BSI, SLECC, AAPI, family conflict, and PSI) across the first 4 visits. Because it was unknown whether illness close to or at the time of the visit would substantially alter the immuneologic outcomes, these multiple regression analyses were repeated including a variable for any report of symptoms of illness in the child within the preceding 14 days. All models used means of stress variables from visits 1 through 4, collected during a 1½-year period, to capture the effect of chronic stress on NK cell function.

The association between short-term changes in stress and NK cell function was tested through analysis of longitudinal data using a mixed model that included random subject effects and serial correlation, determined by the use of the semivariogram. The outcome variable was NK cell function at each of the 4 visits. Factor values were calculated at each visit using the factor loadings based on the means of the psychosocial measures across the 4 visits. This analysis captured the effects of short-term stress and changes in stress levels. Consistent with our analytic approach, a separate model was run using the individual stress variables at each visit as independent variables. In each analysis, NK cell function was log transformed for normality. Linear regression was used to estimate the mean change in the natural log of NK cell activity with a 1-U change in each stress variable.

RESULTS

COHORT DESCRIPTION

Participants included 169 children and their parents (Table 1). The mean age was 7 years for children and 35 years (range, 21–73 years) for their parents; 158 (93.5%) of parents were female; and 151 (89.4%) were mothers of participants. At enrollment, the median score for factor 1 was −0.23 (interquartile range, −0.74 to 0.57), whereas the
The number of child illnesses reported by parents during the 365 days following visit 2 ranged from 0 to 10 (median, 2) (Table 2). The number of febrile illnesses reported during the year following visit 2 ranged from 0 to 6 (median, 0). During the follow-up, 56.2% of the children did not have a febrile illness, 37.3% had 1 or 2, and 7.1% had 3 or more. Of the 389 parental reports of illness, 281 were nonfebrile. The recorded temperatures for febrile illnesses ranged from 38.1°C to 40.5°C (mean, 38.8°C). Parents reported 37 (34.3%) of 108 illnesses as febrile, but did not provide a temperature reading. Although parents indicated that an additional 12 illnesses were febrile, the recorded temperatures were 38°C or lower; these were recorded as nonfebrile illnesses for analysis. Eighty-nine percent of the families had complete diary data for more than 350 days during the year following visit 2.

For each 1-U increase in stress factor 1 (parent impaired by family stress), children had an increased rate of total illnesses of 11% (P = .05; rate ratio, 1.11; 95% CI, 1.00-1.22) and a 36% increased rate of febrile illnesses (P = .001; rate ratio, 1.36; 95% CI, 1.13-1.64) in the subsequent year (Table 3). Analyses with individual stress variables showed that children with parents reporting more psychiatric symptoms (higher BSI scores) also had more illnesses overall (P = .01; rate ratio, 1.49; 95% CI, 1.12-1.97).

### STRESS AND IMMUNOLOGIC ASSESSMENTS

During the first 4 visits, 626 NK cell assays were performed, and 61 (9.7%) of these were excluded owing to insufficient data. Median NK cell function varied from 4.3 to 4.6 across the 4 visits, with an overall range from 0.4 to 4.4.2. The intraclass correlation coefficient of NK cell function in this group of children was 0.4, suggesting that NK cell cytotoxicity assays are a dynamic measure of immune function that have the potential to vary over time within subjects.

For each 1-U increase in factor 1 (parent impaired by family stress), the natural log of NK cell function at visit 4 increased by 0.15 (P = .004; 95% CI, 0.05-0.26). One
individual variable was significantly associated with NK function at visit 4: more family psychosocial adversities (mean score on the SLECC from visits 2-4) were associated with increased NK cell function ($P = .04$; estimate, $0.02; 95\% \text{ CI}, 0.00-0.05$). Results were similar when the child’s illness at visit 4 was included in the model. Sex did not moderate any of these findings (Table 4).

In the longitudinal analysis of NK cell function with stress measures entered separately for each 6-month interval, neither stress factor was significantly associated with NK cell function. However, elevated parental BSI scores were associated with higher NK function ($P = .04$; estimate, $0.22; 95\% \text{ CI}, 0.07-0.38$), and elevated family conflict was associated with lower NK function ($P = .04$; estimate, $-0.01; 95\% \text{ CI}, -0.03$ to $0.00$).

The Poisson regression analyses examining the associations between illnesses and chronic stress were repeated with the addition of the average of NK cell function before and during measurement of illnesses. The NK cell average functions from visits 1 and 2 and from visits 3 and 4 were not associated with illnesses and did not enhance estimates of total illnesses. The addition of NK function also did not reduce the association between stress and illnesses or febrile illnesses. When the Poisson regression analyses were repeated without the 2 stress factors, neither NK cell variable was associated with total illnesses or febrile illnesses.

### Table 4. Adjusted Mean Differences in the Natural Log of NK Cell Function for Study Variables*

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<th>Factor or Variable</th>
<th>Analysis With Factors (n = 137)</th>
<th>Analysis With Variables (n = 137)</th>
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<tr>
<td></td>
<td>Estimate (95% CI)</td>
<td>P Value</td>
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<td>Parent impaired by family stress</td>
<td>0.15 (0.05 to 0.26)</td>
<td>.004</td>
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<tr>
<td>Parenting role stress</td>
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<td>.76</td>
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<td>BSI</td>
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Abbreviations: AAPI, Adult-Adolescent Parenting Inventory; BSI, Brief Symptom Inventory; CI, confidence interval; NK, natural killer; PSI, Parenting Stress Inventory; SLECC, Stressful Life Events and Conditions Checklist; ellipses, not applicable.

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

Children in this cohort had higher rates of illness and febrile illness in families with more chronic stress. Elevated parental psychiatric symptoms occurring in the context of more family stress events (eg, violence exposure and parental unemployment) was identified as a specific dimension of chronic family stress associated with increased illnesses in this cohort of socioeconomically and racially diverse school-aged children. Because of the documented potential for children’s illnesses to increase family stress, this study recorded child illnesses during a 1-year period following the assessment of parental and family stress. Our prospective design strengthens the present findings by reducing the potential for illnesses to confound our measures of family stress.

This study also showed that the combination of elevated parental psychiatric symptoms and family stress events in a 1½-year period was associated with alterations in children’s immune function, expressed by increased NK cell cytotoxicity. By linking elevated parental psychiatric symptoms with both increased illnesses and alterations in children’s immune function, these findings add to the evidence suggesting that chronic family stress has the potential to alter children’s developing immune functions and to have a detrimental effect on health. In analyses using parental stress measures at each 6-month interval, an association between short-term stress and NK cell function was not evident, suggesting that family stress extending for longer periods may have a more consistent influence on immune functions in children.

Elevated parental psychiatric symptoms, as expressed by parental self-report of negative mood and/or dysfunctional behaviors, were associated with higher rates of child illnesses and alteration in NK cell function. These findings are novel because they suggest that impairment in parents’ mood and behavior may be an active mechanism in promoting deleterious effects on children’s health. Previous research has identified impaired parental function as a salient mechanism linking family stress to negative effects on children’s emotional and social functioning. The present findings broaden that evidence to include a possible negative effect on children’s immunity and susceptibility to illness.

Numerous studies of adults have shown diminished NK cell activity during chronic stress. In contrast, we detected increased NK cell function in children from families with high chronic stress. A meta-analysis involving more than 18,000 subjects reported that decreases in NK cell cytotoxicity to naturalistic stress or nonspecific life events were

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stronger for older adults, especially those older than 55 years, suggesting that diminished NK cell function associated with stress may be age dependent and only present in older adults. Our finding is also in keeping with a previous small study of adolescents with major depression that demonstrated an increase in NK cell activity in study subjects compared with nondepressed control subjects.

Stress-illness relationships were not associated with stress-related alterations in NK cell function in this cohort of children. Levels of NK cell function assessed before and during the measurement of illnesses were also not associated with rates of illnesses. It is not known whether other, as yet unspecified, deficiencies in immune function promote a compensatory response of increased NK cell activity.

This study has some limitations. Children’s illnesses were recorded by parents without physician or laboratory confirmation. In addition, despite the provision of a thermometer to all families at enrollment, not all parents recorded temperatures consistently. However, most parents did consistently document their child’s temperature at the time of illness symptoms, and the fact that the number of recorded illnesses during a 1-year period in this group of children was generally low and in keeping with those in the published literature increases our confidence in the reliability of the reporting. Although it is possible that psychiatric distress in parents would increase inaccurate recording of illnesses in their children, we consider it unlikely that parental distress could affect the objective finding of fever. We also note that our cohort had a large proportion of low-income families by recruitment initially through emergency department and other clinic visits, and it cannot be assumed that these findings apply to children in all life contexts.

Although our protocol for blood sampling was designed to minimize acute stress, we cannot exclude venuipuncture as a complicating effect on NK cell function. Most studies of acute stressors demonstrate an increase in NK cell activity. Studies of acute stress typically consist of a laboratory task administered in 15- to 20-minute blocks with blood sampling immediately after completion. In our study, the children were familiar with the study team and protocol and the blood was obtained after quiet play and the use of anesthetic cream. Because of these differences in design, we believe our protocol minimized the effect of acute stress on the results.

CONCLUSIONS

This prospective study found that children of parents with higher levels of psychiatric symptoms in the context of family stressors had more illnesses and febrile illnesses during a 1-year period. Children living in homes with elevated chronic stress also had increased NK cell cytotoxicity. However, alterations in NK cell function were not associated with rates of illness. Further studies of children using more in-depth measures of the psychological-behavioral interaction between parents and their children are necessary to elucidate the specific mechanisms linking family stress with children’s health. In addition, future investigations using more refined indexes of specific aspects of children’s immune function and the types of illnesses responsible for the increase in disease burden need to be performed.

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Author Contributions: Dr Caserta had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Wyman, Moynihan, Cox, Cross, Jin, and Caserta. Acquisition of data: Wyman, Cross, Jin, and Caserta. Analysis and interpretation of data: Wyman, Moynihan, Ebery, Cox, Cross, Jin, and Caserta. Drafting of the manuscript: Wyman and Caserta. Critical revision of the manuscript for important intellectual content: Wyman, Moynihan, Ebery, Cox, Cross, Jin, and Caserta. Statistical analysis: Ebery and Cox. Obtained funding: Wyman, Moynihan, and Caserta. Administrative, technical, and material support: Cross and Caserta. Study supervision: Wyman, Cross, Jin, and Caserta.

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