Double-Exposure to Acute Stress and Chronic Family Stress is Associated With Immune Changes in Children With Asthma

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Objective: To understand how psychological stress heightens risk for asthma flare-ups, we examined the relationship between acute stress, chronic family stress, and the production of asthma-related cytokines. **Methods:** Seventy-one children with asthma and 76 medically healthy children completed interviews regarding life stress, and peripheral blood samples were collected. After mononuclear cells had been mitogenically stimulated, production of the cytokines interleukin (IL)-4, IL-5, IL-13, and IFN-γ was measured. All measurements were repeated every 6 months for 2 years. Children reported on their asthma symptoms for 14 days after each study visit. **Results:** Children with asthma who had higher levels of chronic family stress showed increased production of IL-4, IL-5, and IFN-γ at times when they had experienced an acute event compared with times when they had not. These stress-related changes did not occur in asthmatic children with lower levels of chronic family stress, or in healthy controls. The combination of acute and chronic stress was also associated with increased asthma symptoms. **Conclusion:** These findings suggest that acute negative life events have a particularly strong impact among a subgroup of children with asthma who are under high chronic family stress. The heightened inflammatory profile in this group suggests an explanation for why children experiencing life stressors are at greater risk for asthma exacerbations. **Key words:** asthma, chronic stress, acute stress, cytokines, children.

IL = interleukin; **IFN** = interferon.

INTRODUCTION

Although it has long been known that stressful experiences can exacerbate symptoms of asthma (1), it has only recently become clear which kinds of stressors pose the greatest risk for patients. In this regard, studies indicate that simultaneous exposure to acute life events and ongoing chronic stressors is particularly detrimental. For instance, in a prospective study of children with asthma, those exposed to high levels of acute and chronic stress showed a three-fold increase in risk for an attack in the 2 weeks that followed (2,3). Apart from the issue of duration, research indicates that the type of stressor is an important determinant of its influence, with family difficulties emerging as particularly detrimental. For example, prospective studies have shown that parental stress at 2 to 3 months of life predicts subsequent wheezing in infancy (4), and that parenting difficulties during the first years of life are associated with the onset and persistence of school-age asthma (5,6).

Given the growing evidence of the role of psychosocial factors on clinical asthma outcomes, researchers are beginning to investigate the mechanisms through which stressful experiences contribute to the development and/or persistence of symptoms (1,7). Previous research has found that when asthmatic patients are exposed to stressors like taking an important examination or living in a low socioeconomic status (SES) environment, they show higher eosinophil counts, greater lymphocyte proliferative responses to allergic triggers, and heightened in vitro production of inflammatory cytokines implicated in asthma such as interleukin (IL)-5 and IL-13 (8–10). Stressors also accentuate the airway inflammatory response to allergen challenges (11). However, the bulk of the research on mechanisms has been cross-sectional, meaning that the temporal ordering and causal direction of these associations remain unclear. To begin resolving some of these issues, the current study followed children with asthma over 2 years, taking repeated measures of life stress and asthma-related immune markers. Immune markers included the in vitro production of asthma-related cytokines including IL-4, IL-5, IL-13, and IFN-γ. This design enabled us to identify within-person changes in cytokine dynamics post exposure to stressful experiences. We expected that children who were simultaneously exposed to acute life events and chronic family stress would show alterations in cytokine production in a direction detrimental to asthma.

METHODS

Patients

The sample consisted of 71 children with asthma and 76 medically healthy children (Table 1). They were recruited from the Vancouver, British Columbia community through advertisements in physicians' offices, newspapers and magazines, and community settings. Children were eligible for the study if a) they were between the ages of 9 and 18 years, b) they were fluent in English, and c) had been free of upper respiratory illness for the past 4 weeks. To be included in the asthma group, children were required to have a physician diagnosis of asthma and be free of other chronic medical illness. Healthy children were required to have a history without chronic medical and psychiatric illness. Information regarding children's medical history was gathered from parents.

Procedures

Children visited the research center accompanied by a parent every 6 months over the course of 2 years. Eighty-nine percent of the children had completed three or four study visits at the time of data analysis; they as well as the other 11% are included in the statistical analyses presented below. Written consent was obtained from the parent, and assent was obtained from the child. A local anesthetic cream (EMLA; AstraZeneca, Wilmington, Delaware) was applied to the child's arm. Children were interviewed regarding life stress, and then a blood sample was collected using antecubital venipuncture. Children with asthma reported on their asthma symptoms twice daily for 14 days after each laboratory visit. Data collection for this portion of the study began in April of 2004 and ended in September of 2007. The study was approved by the Research Ethics Board of the University of British Columbia.

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TABLE 1. Demographic and Health-Related Characteristics of Participants

Characteristic	Statistic	
	Asthma (n = 71)	Healthy (n = 76)
Age (years)	12.77 ± 2.68	12.89 ± 2.34
Sex (males), %[a]	72	52.6
Ethnicity (White), %	63	63
Annual family income (>$50 000), %	81	69.5
Asthma severity, %		
Mild intermittent	16.9	—
Mild persistent	38.0	—
Moderate	32.4	—
Severe	12.7	—
Diary symptoms[b]	0.71 ± 0.74	—
Atopic asthma, %	84%	—
B-agonist use, %[c]	83	—
Inhaled corticosteroid use, %[c]	65	—
Chronic family stress[d]	2.23 ± 0.66	2.38 ± 0.84
Acute events, %[d]	16–19	18.9–31.1
IL-4 production (pg/ml)	6.79 ± 5.53	9.02 ± 9.77
IL-5 production (pg/ml)	81.90 ± 82.69	91.13 ± 142.46
IL-13 production (pg/ml)	285.36 ± 194.84	317.64 ± 259.09
IFN-γ production (pg/ml)	12373.45 ± 16211.94	13930.88 ± 12797.94

Values represent mean ± standard deviation. Means for cytokine production and diary symptoms were averaged across study visits. We compared the 11% of participants who had only completed two study visits to the larger sample and found no significant differences in cytokine production, but significantly lower acute and chronic stress. Nevertheless, the group with more complete data (the 89% who had completed three or four study visits) did still represent the full range of scores on chronic family stress.

[a] Groups were statistically different ($p < .05$) according to χ^2 test.

[b] Ratings of asthma symptoms were made on a scale from 0 (none) to 4 (really bad). Morning and evening ratings were summed, and these scores were averaged across the 14 days of sampling.

[c] Coded as whether or not the child had been prescribed the medication.

[d] The average chronic family stress rating corresponds to a mild to moderate level of family stress. An acute event was defined as an event rate ≥2.5. Here we report the range of frequencies of acute events across visits.

IL = interleukin; IFN = interferon.

Life Stress Interview

To assess children's exposure to stressful experiences, we administered the University of California Los Angeles Life Stress Interview, Child Version (12). This semistructured interview covers acute and chronic forms of stress over the past 6 months. It has been used successfully in children as young as 8 years old, and has demonstrated reliability and validity (13–15). The interview focuses on stress in multiple life domains, including family relationships, friendships, school, and home life. Interviewers rate the level of chronic, ongoing stress on a 5-point scale, with higher numbers reflecting more severe and persistent difficulties. Here we focus on chronic family stress, which is concerned with the quality of interpersonal relationships among family members. Chronic family stress was quite stable over time (Cronbach's α = 0.90; intercorrelations range = 0.61–0.84); thus, we could not justify modeling it within-person. More specifically, the average change across time per subject was about a half point, which is actually within our margin of error across different raters. Thus, chronic family stress ratings were averaged across the four visits and modeled as a between-subjects factor.

The interview also probed for acute stressors, defined as specific events with a discrete onset and offset. To judge the objective impact of an acute event, a consensus rating is made by a team of interviewers after it has been briefed on event details by the primary interviewer. Impact ratings are made on a 5-point scale ranging from 1 (no impact) to 5 (severe impact). Consensus ratings take into account the context in which an event has occurred. For example, if a participant had failed a test at school, we would make a rating based on a number of factors, such as his or her previous academic record, and reactions from parents and teachers. In the present study, we consider acute events rated ≥2.5 (mild-moderate impact) to be significant life events. This follows the convention in the Life Events and Difficulties Schedule area of using a threshold for defining major life events (16–18). Examples of acute life events from the current study include a mother being fired from her job, a father being admitted to the hospital for depression, and a child's best friend moving to another city.

Cytokine Production

Cytokine release by peripheral blood mononuclear cells (PBMC) in response to mitogenic stimulation was measured in an in vitro model. Peripheral blood sample was collected into BD Vacutainer Cell Preparation Tubes containing sodium heparin, and PBMCs were separated and stimulated with phorbol myristate acetate (PMA) (final concentration = 25 ng/ml) and ionomycin (final concentration = 1 μg/ml) for 48 hours at 37°C in 5% CO_2. This PMA/ionomycin combination has been successful in stimulating the cytokines of interest in other asthma studies (19,20). After centrifugation, supernatants were collected and frozen at −80°C. Supernatants were then assayed to determine levels of IL-4, IL-5, IL-13, and IFN-γ, using enzyme-linked immunosorbent assays (R&D System, Minneapolis, MN). Inter- and intra-assay variations were <10%.

Asthma Symptoms

Children rated each of four asthma symptoms (cough, wheeze, chest tightness, and shortness of breath) on a scale from 0 (none) to 4 (really bad). Symptoms experienced over the course of the night were rated at waking and symptoms experienced during the day were rated before bed on each day of sampling. Compliance to the diary schedule was monitored using electronic time and date stamps that the participants would "punch-in" before making their ratings. To create symptom scores for each visit, we computed average daily symptoms scores, and then averaged the daily scores across the 14 days.

Potential Confounders

To account for the impact of medication, parents were asked to bring all of their child's medications to the research center. Names and dosages of medications were recorded directly from the label, and the number of days each medication was taken in the last 2 weeks was ascertained. Using this information, we created variables reflecting the number of days in which inhaled corticosteroids and β agonists were used in the past 2 weeks.

Asthma severity was determined from the National Asthma Education and Prevention Program, Expert Panel Report 2 guidelines based on the higher of symptom frequency and medication use (21). Symptom frequency was based on parent reports of the child's daytime, nighttime, and exertional symptoms over the past month. Medication use and asthma severity were included as covariates in statistical analyses.

Finally, SES as a possible covariate was measured, using current family income. Current family income was defined as the family's total gross income for the past 12 months before taxes.

Statistical Analyses

Data were analyzed using hierarchical linear modeling (HLM 6.03) (22), a multilevel modeling technique that allowed us to test both within-person and between-person contributions to changes in cytokine production and asthma symptoms over time. Acute stress was modeled as a within-person factor because it varies over time, whereas chronic family stress was modeled as a between-subjects factor because it varies from child to child but remains stable over time. In the within-person (or level-1) model, we estimated cytokine production as a function of factors that vary over time including

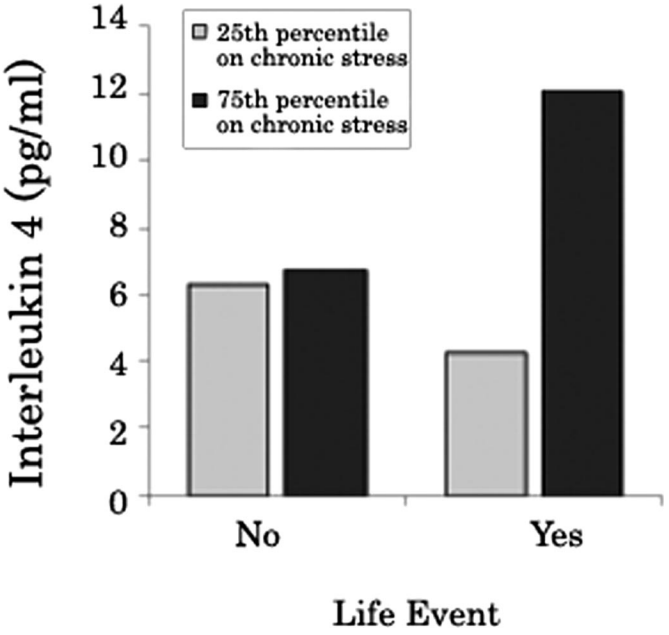

Figure 1. Predicted values of stimulated interleukin-4 at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event ($n = 71$).

exposure to a significant life event and the covariates of days of medication use in the last 2 weeks. These models yielded a series of person-specific slopes reflecting the difference in cytokine production between visits when patients were versus were not exposed to a significant life event (β_{1i}). In the between-person (or level 2) models, we estimated the above slopes for each subject as a function of factors that vary across people, including chronic family stress and the covariate asthma severity. The critical parameter was the coefficient γ_{11}, which reflects the cross-level interaction between acute stress and chronic stress in the prediction of cytokine levels. In other words, the coefficient γ_{11} indicates whether the relationship between acute stress and cytokine production depended on the extent of chronic family stress. When significant interactions emerged, cytokine production was plotted at the 25th and 75th percentiles of chronic family stress in both the absence and presence of an acute event. In addition, the magnitude of the interactions was examined by calculating the proportion of between-person variability in the relationship between acute stress and cytokine production that was explained by chronic family stress.

RESULTS

Relations of Life Stress to Cytokine Production Among Patients with Asthma

First, we tested the main effects of acute stress at level 1. Results indicated no significant effects for any of the outcome measures ($p > .10$), meaning that patients had similar patterns of cytokine production at times when they had experienced an acute event compared with times when they had not. We then tested the main effects of chronic stress at level 2, and again, results indicated no statistically significant effects ($p > .05$). However, there was a trend toward a positive relationship between chronic family stress and IL-4 production, $\gamma_{01} = 1.84$, SE = 0.96, $p = .06$. Specifically, children with higher family chronic stress showed marginally higher IL-4 levels.

Next, we tested for a level 1 by level 2 interaction, in terms of whether the relationship between acute stress and IL-4 production depended on the extent of chronic family stress. Results indicated a significant interaction between acute and chronic stress ($\gamma_{11} = 8.16$, SE = 3.15, $p = .01$). Consistent with expectations about the detrimental nature of double-exposure, children with higher levels of chronic family stress showed increased IL-4 at times when they had experienced an acute event compared with times when they had not (Figure 1). In contrast, children with lower levels of chronic family stress showed no changes in IL-4 at times when they had experienced an acute event compared with times when they had not. The moderating effect of chronic stress was large, as it accounted for approximately 77% of the between-person variability in the relationship between IL-4 and acute events.

We found similar cross-level interactions for IL-5, $\gamma_{11} = 43.74$, SE = 15.46, $p = .007$ and IFN-γ, $\gamma_{11} = 11334.98$, SE = 3803.80, $p = .004$ (Table 2; Figures 2 and 3). Children with higher levels of chronic family stress showed increased IL-5 and IFN-γ production at times when they had experienced an acute event compared with times when they had not. Conversely, children with lower levels of chronic family stress showed no difference in production of these cytokines at times when they had experienced an acute event compared with times when they had not. Chronic stress accounted for approximately 73% of the between-person variability in the relationship between acute events and each of these outcomes.

In contrast to our other findings, acute and chronic stress did not interact in the prediction of IL-13 production ($p > .20$).

To test whether these findings could be explained by family SES, we reran analyses including family income as a control variable at level 2. Acute stress and chronic family stress continued to interact in predicting IL4, IL5, and IFN- γ production, independent of family income ($p < .05$).

Analyses were also repeated, using a 6-month (rather than 3-month) window for major life events. However, these analyses indicated no significant main effects of acute stress ($p > .10$) and no significant acute by chronic interactions ($p > .20$). Thus, only more recent events (within the 3 months leading up to each visit) seemed to interact with chronic stress in the prediction of IL-4, IL-5, and IFN-γ.

Relations of Life Stress to Asthma Symptoms

We next asked whether chronic and acute stress would predict asthma symptoms. These analyses were carried out in a subgroup of participants with moderate-to-severe asthma ($n = 32$) because they were the only ones to report symptoms consistently. The mean symptom score for this subgroup was 1.04 ± 1.01 on a scale of 0 to 4; in the rest of the sample, it was 0.71 ± 0.81.

Analyses indicated that, whereas there were no main effects of acute or chronic stress on symptoms ($p > .20$), there was a significant cross-level interaction ($\gamma_{11} = 0.41$, SE = 0.17, $p = .024$) (Table 2). As shown in Figure 4, the pattern of findings for asthma symptoms partially mirrored the pattern for cytokine production. Specifically, as was the case with

TABLE 2. Hierarchical Linear Models Testing the Effects of Acute Stress, Chronic Family Stress, and the Acute Stress by Chronic Stress Interaction on Cytokine Production and Asthma Symptoms

	Asthma[a]				Healthy			
Model	Coefficient	SE	*p*	Effect Size (%)	Coefficient	SE	*p*	Effect Size (%)
IL-4								
Acute	0.083	2.15	.97	—	2.91	2.74	.29	—
Chronic	1.84	0.96	.06	—	−0.55	0.88	.54	—
Interaction	8.16	3.15	.01	77	−4.83	3.59	.18	—
IL-5								
Acute	4.98	14.75	.74	—	58.01	24.80	.02	—
Chronic	13.96	12.46	.27	—	−13.42	18.59	.47	—
Interaction	43.74	15.46	.007	73	−22.18	19.26	.25	—
IL-13								
Acute	−21.87	36.66	.55	—	21.06	76.25	.78	—
Chronic	41.83	29.93	.17	—	−73.45	29.38	.02	—
Interaction	15.94	36.81	.67	—	20.00	88.51	.82	—
IFN-γ								
Acute	3160.30	3818.92	.41	—	−2493.53	4005.10	.54	—
Chronic	3024.24	2186.88	.17	—	1491.05	1370.62	.28	—
Interaction	11334.98	3803.80	.004	72	−7502.52	4261.56	.08	—
Symptoms[b]								
Acute	0.14	0.16	.41	—	—	—	—	—
Chronic	0.33	0.25	.20	—	—	—	—	—
Interaction	0.41	0.17	.024	88	—	—	—	—

The effect size measure is the percentage of the between-person variability in the relationship between acute stress and the outcome that is accounted for by chronic family stress.

[a] Models testing the main effect of chronic stress and the interaction between acute and chronic stress statistically control for asthma severity and medication use. Models testing the main effect of acute stress statistically control for medication use.

[b] The effect of life stress on symptoms was tested in a subgroup of children with moderate-to-severe asthma ($n = 32$).

IL = interleukin; IFN = interferon.

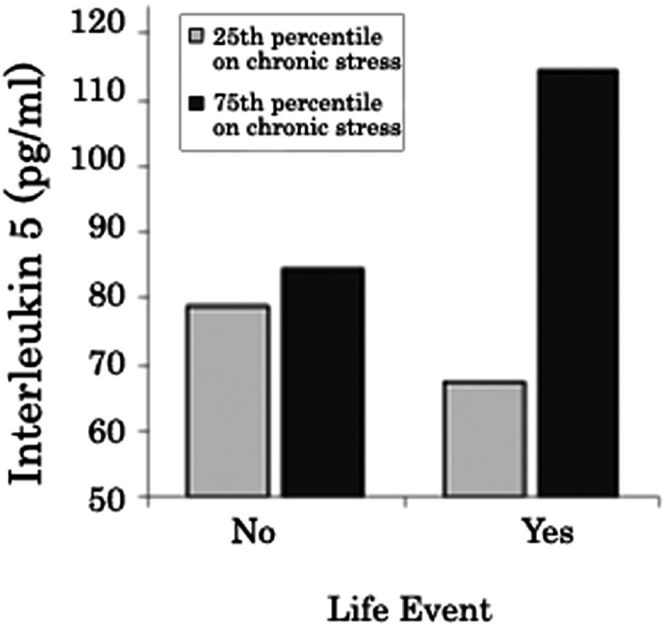

Figure 2. Predicted values of stimulated interleukin-5 at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event ($n = 71$).

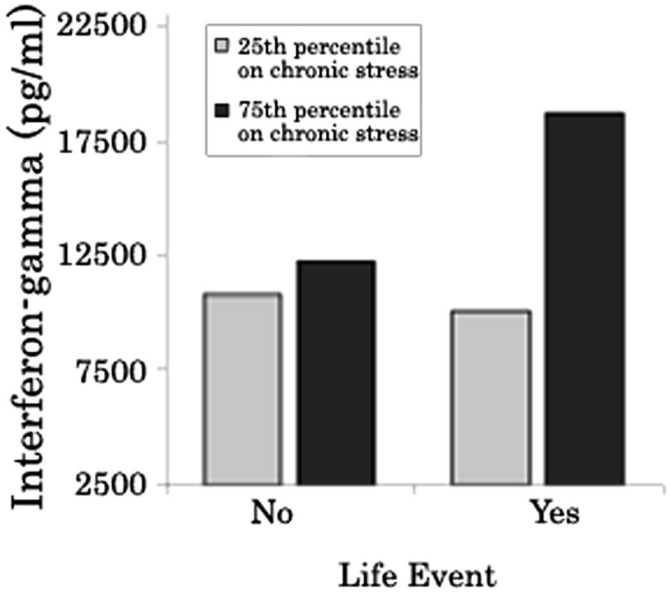

Figure 3. Predicted values of stimulated interferon-γ at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event ($n = 71$).

cytokines, the impact of acute events was amplified among children with high chronic family stress, such that patients with simultaneous exposure to these stressors reported the most symptoms. But unlike the cytokine outcomes, there was evidence that chronic family stress increased symptoms even at times when no acute event had occurred (although this amplifying effect of chronic stress was smaller than at times when an acute event had occurred). The cross-level interaction

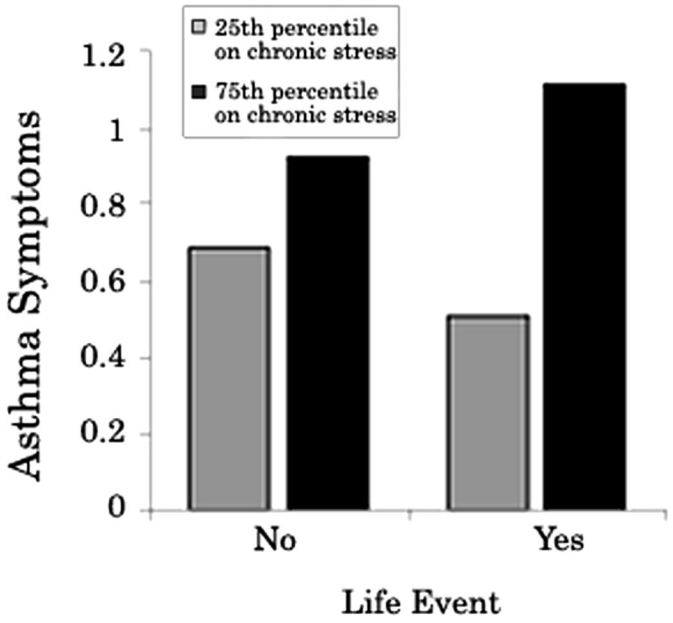

Figure 4. Predicted symptom scores at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event. These data represent a subgroup of children who have moderate to severe asthma ($n = 32$).

explained approximately 88% of the between-person variability in the relationship between acute stress and asthma symptoms.

Relations of Cytokine Production to Asthma Symptoms

Given that life stress was associated with IL-4, IL-5, and IFN-γ production, we tested whether within-person changes in these cytokines were associated with changes in asthma symptoms. This set of analyses was also carried out among the subgroup of asthma participants with moderate-to-severe symptoms. Results indicated that IL-5 production was significantly associated with asthma symptoms ($\gamma_{01} = 0.0024$, SE = 0.00067, $p = .002$), meaning that participants reported increased asthma symptoms at times when IL-5 production was higher than their individual averages. In addition, there was a trend for a positive association between IL-4 production and asthma symptoms ($\gamma_{01} = 0.011$, SE = 0.0055, $p = .06$). However, IFN-γ production was unrelated to asthma symptoms ($p > .30$).

Relations of Life Stress to Cytokine Production Among Healthy Controls

We conducted a parallel set of analyses in the healthy comparison group (Table 2). They showed that exposure to an acute event (within 3 months) was associated with increased production of IL-5 ($\gamma_{01} = 58.01$, SE = 24.80, $p = .02$), meaning that participants showed increased production of IL-5 at times when they had experienced an acute event compared with times when they had not. However, there were no main effects of acute events on IL-4, IL-13, or IFN-γ ($p > .30$). The analyses also revealed a significant main effect of chronic family stress on IL-13, such that children with higher levels of chronic family stress showed increased production of IL-13, $\gamma_{01} = -0.73.45$, SE = 29.38, $p = .02$. There were no main effects of chronic family stress on production of IL-4, IL-5, or IFN-γ, $p > .30$. Finally, we tested the interaction between acute and chronic stress. Unlike the asthma sample, results indicated that acute and chronic stress did not interact to predict IL-4, IL-5, or IL-13, $p > .10$. However, there was some trend toward an interaction between acute and chronic stress in the prediction of IFN-γ, $\gamma_{01} = -7502.52$, SE = 4261.56, $p = .08$.

DISCUSSION

This study examined the relationship between chronic stress, acute events, and within-person changes in cytokine production over 2 years in children with asthma and a healthy comparison group. Within the asthma group, we found that the association between acute events and asthma-relevant cytokines was accentuated among children with chronic family stress. Specifically, children with high chronic family stress showed increased production of IL-4, IL-5, and IFN-γ at times when they had experienced an acute event compared with times when they had not. In contrast, when chronic family stress was low, there was no relationship between acute events and cytokine production. Acute and chronic stress did not interact to predict cytokine changes among medically healthy children, suggesting that the cytokine changes are specific to asthma and not simply part of a general response to stress. Furthermore, in a subgroup of children with moderate-to-severe asthma, acute and chronic stress interacted to predict asthma symptoms, such that children who were exposed to both acute and chronic stress reported the most severe symptoms. This pattern of results mirrors previous clinical findings in which double-exposure to acute and chronic stressors was associated with risk for asthma exacerbations (2). The present study takes this research one step further by providing a plausible biological explanation for how a psychological factor like stress can influence clinical outcomes in asthma.

One reason why double-exposure may be so detrimental is that it may be that, although people can manage one key life stressor, they do not have the resources (e.g., time, energy, social support) to deal effectively with multiple stressors. This might prolong the impact or heighten the severity of stressors that could normally be managed. Having to juggle multiple stressors also seems likely to interfere with people's ability to engage in healthy behaviors like exercising and spending time with friends that can protect against the detrimental influence of problems at home, work, and school. Double-exposure may also overwhelm a person's social support network or, if one of the stressors involves tensions with close friends or family, interfere with his or her ability to mobilize it. Erosion of family support could be especially detrimental for children, who often rely on their parents and siblings for assistance with key life events.

What are the mechanisms by which double-exposure influences cytokine production? One possibility is that stress-

related activation of the hypothalamic-pituitary-adrenocortical axis and the sympathetic nervous system among double-exposed children leads over time to a compensatory downregulation of glucocorticoid and β-adrenergic receptors on target tissues. This process is thought to decrease sensitivity to the anti-inflammatory effects of hormones like cortisol and epinephrine (18,23). Consistent with this theory, double-exposure has been linked to elevated cortisol output through the day and decreased expression of glucocorticoid receptor mRNA among healthy young women (24) as well as decreased expression of both glucocorticoid and β-adrenergic receptor mRNA among youth with asthma (18). Thus, the present findings of heightened cytokine production may, in part, result from diminished control over inflammation due to dysregulation at the level of hormones and receptors. Future work would benefit from prospective studies that examine the causal relations between stress exposures, hormone dynamics, and asthma-related immune markers.

This study examined T-helper (T_H) 2 (IL-4, IL-5, and IL-13) and T_H1 (IFN-γ) cytokines, which serve different functions during an immune response. In regard to asthma, it is widely believed that the inflammatory response involves a T_H2 mechanism (25–27). In addition, there is some evidence that acute stress involves a shift toward a T_H2 response (increased production of IL-10) and away from a T_H1 response (decreased production of IFN-γ) among healthy young adults (28). Consistent with these findings, double-exposed children with asthma in our sample showed increased T_H2 cytokine (IL-4 and IL-5) production, which could be indicative of an enhanced humoral response to allergens. However, they also showed increased production of IFN-γ, a T_H1 cytokine that is thought to have inhibitory effects on T_H2 pathways. These results are consistent with some previous research—for example, the finding that another type of chronic stress, low SES, is associated with elevations in both T_H2 (IL-5) and T_H1 (IFN-γ) responses among children with asthma (29). Thus, it may be that, in children with asthma, stress leads to a general upregulation of T_H cell activity and signaling (rather than an imbalance between T_H1 and T_H2). What this means for asthma is unclear. Although Th1 activity generally inhibits the Th2 signals that drive asthma, some data suggest that cytokines like IFNg contribute to asthma pathogenesis, possibly by orchestrating antiviral immune responses in the lungs (30). If this is the case, stress-related priming of IFN-g responses could have detrimental influences on asthma symptoms, especially when they have been triggered by viral infection (1,31).

This study showed that, among children with moderate-to-severe asthma, acute stress and chronic family stress interacted to predict asthma symptoms. The pattern of the interaction was similar to the interaction of acute and chronic stress predicting IL-4, IL-5, and IFN-γ production. However, in addition to the pattern found with cytokines, chronic family stress also seems to influence asthma symptoms in the absence of an acute event. Specifically, when no event had occurred, chronic family stress was positively associated with asthma symptoms, but to a lesser degree than after an acute event. The difference in patterns for cytokine production versus symptoms suggests that there are pathways in addition to immune changes by which chronic family stress can influence symptoms (32).

Are the observed increases in IL-4, IL-5, and IFN-γ production really relevant to asthma? To test this question, we also examined the relationship between cytokine production and asthma symptoms among subjects with higher severity asthma. Results indicated that increased production of IL-5 was associated with within-person increases in asthma symptoms, and there was a trend toward a similar effect for IL-4 production. This provides evidence that changes in IL-5 and IL-4, but not IFN-γ, production are relevant to asthma symptoms.

This study has a number of limitations. First, it is possible that unmeasured variables account for our effects. For instance, increased risk for infection after life events could explain the relationships of acute events and cytokine production. However, if this were the case, one would expect to see effects of infection on both the main effect of stressful events as well as the interaction between acute and chronic stressors. Moreover, the within-person nature of our analyses and findings precludes most of the alternative explanations for linkages between stressors and immunity (e.g., genetics, lifestyle factors). Second, it will be important to see how factors like gender and puberty moderate the effects of double-exposure on cytokine production; however, in the present study, we did not have enough power to test these three-way interactions. Third, our inability to detect main effects of acute events may reflect the fact that cytokine production was not always assessed shortly after the stressor occurred, when they would be most likely to emerge. Future research that examines inflammatory processes within weeks of the stressful event may be more likely to detect such findings. Fourth, immune processes were measured from peripheral blood cells. Future studies that are able to obtain cells directly from the airways would provide important information about more proximal processes. Fifth, the cytokine production assay used a substance that stimulates cells nonspecifically, rather than through the antigen-specific pathways that are engaged by most allergens in the body. However, given that our sample was not selected for a positive response to any specific allergen, the use of a specific stimulus would not have elicited responses across all children. Finally, it should be noted that youth were recruited into this study on the basis of parent-reported physician diagnosis of asthma and that physician diagnosis is not always accurate. However, if we inadvertently included some children who did not truly have asthma, this in theory would add noise to our measurements, making it more difficult to detect associations.

Despite these limitations, our findings provide several important contributions to the literature. First, they suggest that stressor impact is a complex phenomenon—the combination of acute and chronic stress may be necessary to produce a change in cytokine production. Second, the immune changes

observed among children who had been exposed to acute and chronic stress are relevant to asthma symptomatology. Third, this type of research contributes to biopsychosocial models that seek to explain how larger social factors can get "under the skin" to affect an individual child's health. The fact that experiencing an acute life event on top of high chronic stress predicts detrimental inflammatory responses suggests that psychosocial interventions aimed at helping children manage stress could have implications biologically for children with asthma.

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