

Chronic caregiver stress and IgE expression, allergen-induced proliferation, and cytokine profiles in a birth cohort predisposed to atopy

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Background: Psychologic stress modifies immune function and cytokine production.

Objective: We examined relationships between caregiver stress on the following markers of early childhood immune response: (1) IgE expression (n = 215); (2) mitogen-induced and allergen-specific (*Dermatophagoides farinae* [Der f 1] and cockroach [Bla g 2]) proliferative response (n = 114); and (3) subsequent cytokine expression (INF- γ , TNF- α , IL-10, and IL-13) in a prospective birth cohort predisposed to atopy.

Methods: Caregiver stress was measured at 2-month intervals for the first 2 years of life and yearly thereafter by using the Perceived Stress Scale. A subsequent blood sample obtained from the children (median age, 2.1 years; range, 18-32 months) was analyzed for total serum IgE level and allergen-induced proliferation quantified as the stimulation index (SI; mean thymidine incorporation of the stimulated sample divided by that of the unstimulated sample). The relationship between stress and the proliferative response (SI >3 vs SI \leq 3), and total IgE level (\leq 100 IU/mL vs >100 IU/mL) was examined by using logistic regression. The relationship between cytokine levels and stress was analyzed by using linear regression.

Results: In adjusted analyses higher caregiver stress in the first 6 months after birth was associated with a Der f 1 SI of greater than 3 (odds ratio [OR], 1.5; 95% CI, 1.0-2.3) and nominally associated with a Bla g 2 SI of greater than 3 (OR, 1.13; 95% CI, 0.7-1.8). Higher stress between ages 6 and 18 months was associated with a high total IgE level (OR, 2.03; 95% CI, 1.1-3.6). Higher stress was significantly associated with increased

production of TNF- α , with a suggested trend between higher stress and reduced INF- γ production.

Conclusion: Increased stress in early childhood was associated with an atopic immune profile in these children predisposed to atopy-asthma. (*J Allergy Clin Immunol* 2004;113:1051-7.)

Key words: Caregiver stress, IgE, lymphocyte proliferation, cytokines, birth cohort

The majority of asthma involves allergen-mediated inflammation, with T-lymphocyte activation central to the allergic asthmatic response.¹ The most common paradigm divides T-helper cells into profiles of differentiated cell function having different patterns of cytokine release on activation (eg, T_H1 and T_H2).² T_H1 cells tend to express IL-2 and INF- γ , and T_H2 cells tend to express IL-4, IL-5, and IL-13.³ Allergy and asthma might also be influenced by cytokines produced through pathways or inflammatory processes not directly related to the T_H1/T_H2 dichotomy.⁴ For example, some proteins are secreted both by T_H1 and T_H2 cells (eg, TNF- α). Studies suggest that TNF- α , produced by both lymphocytes and macrophages, is involved in the atopic response.^{5,6} Activation of T_H2 lymphocytes underlying allergen sensitization is associated with increased IgE production.⁷ Seroepidemiologic studies have shown an association between increased total IgE levels and asthma that increases in magnitude and significance over the first 4 to 6 years of life.⁸

Environmental exposures might influence early immune function and cytokine production. Polarization of the immune system into an atopic phenotype likely occurs during early childhood and even before birth.⁹ For example, studies suggest that maternal exposure to inhaled allergens can prime fetal T cells toward an atopic phenotype.^{10,11} Our laboratory has demonstrated that allergen exposure at 3 months of age predicts allergen-specific lymphocyte proliferative responses at age 2 years.¹²

Exposure to other environmental factors at critical developmental periods that induce functional changes in neuroendocrine and immune processes might also polarize the immune system toward an atopic phenotype.^{13,14} Dysregulation of homeostatic neuroimmunologic mechanisms can occur in the face of chronic stress that affects cytokine expression, allergic inflammation, and asthma expression. The family is the most important social context influencing the health and development of

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Abbreviations used

OR: Odds ratio
PSS: Perceived Stress Scale
SI: Stimulation index

children, with family functioning and interactions with a primary caregiver being important sources of stress.¹⁵ Growing evidence links caregiver stress to the child's stress response. Maternal stress influences the hormonal stress response in early childhood and modifies infant neuroendocrine function during development, even beginning prenatally.^{16,17} Investigators have demonstrated an increased cortisol response in children from families that show high stress.¹⁸

We examined the influence of caregiver stress beginning in the first 2 to 3 months of the index child's life on the lymphocyte proliferative response, cytokine production, and total IgE expression in a population predisposed to atopy-asthma. We hypothesized that higher caregiver stress would be associated with an enhanced inflammatory response, including an increased allergen-specific proliferative response and higher levels of IgE expression.

METHODS

Participants in the Home Allergen and Asthma Study include infants and their 499 families with a history of asthma or allergy recruited within 48 hours of delivery between September 1994 and July 1996, as previously reported.¹⁹ The Brigham and Women's Hospital Human Studies Committee approved the study, and written parental informed consent was obtained.

At the home visit, when the index child was 2 to 3 months old, data were collected on environmental exposures, including dust samples analyzed for *Dermatophagoides farinae* (Der f 1) and cockroach (Bla g 1 or 2), as previously described.¹⁹ Quantification of allergen was performed by means of 2-site monoclonal and polyclonal antibody immunoassays.²⁰ Der f 1 concentrations were reported as micrograms of allergen per gram of dust, whereas Bla g 1 or 2 concentrations were reported as units per gram of dust. A home maximum was determined on the basis of the highest allergen level obtained from sampling in the index home. Data on sociodemographic factors were also obtained through personal interview questionnaires.

Standard control variables

The child was classified as white if both parents were white, black if either parent was black, Hispanic if no parent was black but at least one parent was Hispanic, and Asian if at least one parent was Asian but no parent was black or Hispanic. Annual household income was stratified as unknown, \$15,000 or less, \$15,000 to \$29,000, \$30,000 to \$49,000, and \$50,000 or greater. Analyses were controlled for child's age and sex and maternal active asthma, as previously defined.¹⁹

Psychologic stress. Data on caregiver stress were obtained at the initial home visit. Subsequently, bimonthly telephone questionnaires ascertained changes in caregiver stress for the first 2 years of life and yearly thereafter. The stress questionnaire was administered to the index child's primary caregiver at each follow-up contact (ie, the same caregiver responded at each interview). Caregiver stress was ascertained by using the 4-item Perceived Stress Scale (PSS),²¹ a measure of the degree to which respondents believed their lives

were unpredictable, uncontrollable, and overwhelming in the preceding 1 month (reliability, 0.85).²² The PSS was developed on the basis of Lazarus's concept of stress appraisal,²³ which is central to current stress theory. When confronting demands, individuals appraise whether the event is threatening or potentially overwhelming to their existing coping resources.²⁴ This perception is presumed to result in negative emotional states (eg, anxiety and depression), which in turn might be accompanied by neuroendocrine and immunologic changes that affect health outcomes. Each item is scored on a 5-point frequency scale, ranging from "never" (0) to "very often," and scores were obtained by summing the 4 items (maximum, 16). The PSS has been validated in a representative population for measuring stress across age groups, race, sex, and socioeconomic status²⁵ and reflects changes in stress over time.²⁶ Studies have shown the prospective relationship between the PSS and multiple behaviors, health outcomes, and cytokine expression.²⁵⁻²⁹ The PSS predicts outcomes independently of psychologic symptoms, showing good discriminant validity.^{25,28}

Total serum IgE levels. Serum samples were analyzed for total IgE antibodies (n = 215), as previously described.³⁰ Results are reported in international units of IgE. We defined a high total IgE value as 100 IU/mL or greater, a level that has been associated with sensitization and subsequent childhood asthma risk.³¹

Proliferative response. A subsample of 114 children was selected for peripheral blood analysis on the basis of home allergen levels¹⁹ and complete data on covariates. Blood was obtained for lymphocyte proliferation when the children presented for state-mandated lead testing at a median age of 2.1 years (range, 18-32 months). At the blood draw, parents were asked to report whether the child had an upper respiratory infection within the previous week.

PBMCs isolated from heparinized blood³² were immediately processed and not cryopreserved. Briefly, cells were incubated for 72 hours with media alone, media with PHA (10 µg/mL), or media with Der f 1 (30 µg/mL) and Bla g 2 (30 µg/mL). After 72 hours, tritiated thymidine uptake was determined by means of β-counting. Proliferation was quantified by using the stimulation index (SI), which was defined as the mean thymidine incorporation of the stimulated sample divided by that of the unstimulated sample. The lymphocyte proliferative response was considered as a binary variable indexed as a high (SI >3) or low (SI ≤3) response.

Allergen exposure. Allergen exposure levels were categorized as binary indices for which associations between exposure and either sensitization or development of allergy-related wheeze has been determined.¹² Der f 1 levels were categorized as follows: high (≥10 µg/g dust), intermediate (2 to <10 µg/g dust), low (<2 µg/g dust), and no dust. Bla g 1 or Bla g 2 levels were categorized as high (≥2 U/g dust), intermediate (0.05 to <2 U/g), low (<0.05U/g), and no dust. Values of greater than the upper levels of detection for the assay were considered high, and those that were less than the levels of detection were indexed as low.

Cytokines. Supernatants from allergen- and mitogen-stimulated samples were harvested at 24 and 60 hours. On the basis of optimization of the detection of cytokine levels, IL-10 and TNF-α secretion were measured in the 24-hour sample, and IFN-γ and IL-13 secretion were measured in the 60-hour sample (Endogen, Cambridge, Mass).¹² Cytokines were analyzed by means of ELISA according to the manufacturer's protocol. Detection limits were typically 1.25 to 2.5 pg/mL.

Analyses

Descriptive summaries, including means and proportions, describe the characteristics of the sample stratified by high or low IgE levels and SIs. Differences across groups were examined by using the Fisher exact test and the *t* test of equality of the means.

TABLE I. Distribution of covariates relative to total IgE level and SI for Der f 1* and Bla g 1 and 2†

Predictor	IgE ≤100 IU/mL, N (%)	IgE >100 IU/mL, N (%)	Der f 1		Bla g 1 or 2	
			SI ≤3, N (%)	SI >3, N (%)	SI ≤3, N (%)	SI >3, N (%)
Sex						
Male	113 (57.4)	12 (67)	33 (66)	37 (58)	20 (66.7)	50 (60.2)
Female	84 (42.6)	6 (33)	17 (34)	27 (42)	10 (33.3)	33 (39.8)
Race						
White	149 (75.6)	14 (78)	34 (68)	50 (78.1)	24 (80)	59 (71.1)
Black	22 (11.2)	3 (17)	4 (8)	7 (10.9)	3 (10)	8 (9.6)
Other	26 (13.2)	1 (5)	12 (24)	7 (10.9)	3 (10)	16 (19.3)
Household income						
>\$50,000	142 (72.8)	10 (56)	34 (68)	45 (71.4)	22 (73.3)	56 (68.3)
\$30,000-\$50,000	33 (16.9)	4 (22)	10 (20)	12 (19.0)	4 (13.3)	18 (22.0)
<\$30,000	19 (9.7)	4 (22)	6 (12)	6 (9.5)	4 (13.3)	8 (9.8)
Unknown	1 (0.5)	0	0	0	0	0
Maternal asthma						
No	156 (79.2)	13 (72)	37 (74)	53 (82.8)	23 (76.7)	66 (79.5)
Yes	41 (20.8)	5 (28)	13 (26)	11 (17.2)	7 (23.3)	17 (20.5)
Cold status						
No	148 (75.9)	12 (66.7)	32 (67)‡	53 (84.0)‡	17 (59)‡	67 (83)‡
Yes	47 (24.1)	6 (33.3)	16 (33)‡	10 (16.0)‡	12 (41)‡	14 (17)‡
Age at blood draw, mean ± SE						
	2.40 ± 0.04	2.22 ± 0.11	2.28 ± 0.07§	2.51 ± 0.07§	2.47 ± 0.10	2.39 ± 0.06
Household maximum						
Der f 1						
Low (<2 µg/g dust)	67 (34)	7 (38.9)	17 (34)	26 (40.6)		
Medium (2-10 µg/g dust)	48 (24.4)	5 (27.8)	15 (30)	10 (15.6)		
High (10 µg/g dust)	82 (41.6)	6 (33.3)	18 (36)	28 (43.8)		
Bla g 1 or 2						
Low (<0.05 U/g)	76 (38.6)	6 (33.3)			16 (53.3)	27 (32.5)
Medium (0.05 to <2 U/g)	96 (48.7)	9 (50)			12 (40)	42 (50.6)
High (2 U/g)	25 (12.7)	3 (16.7)			2 (6.7)	14 (16.9)

**Dermatophagoides farinae* (Der f 1) dust mite antigen.

†Cockroach (Bla g 1 or Bla g 2).

‡Fisher exact test of homogeneity of proportions, *P* < .05.

§t Test of equality of the means, *P* < .01.

These data have a nonstandard structure in that the caregiver stress covariate is longitudinal for each family (repeated and measured at irregular time points approximately every 2 months), whereas the immune responses (IgE, SI, and cytokines) are measured at a single time point for each child. We were interested in the association between different profiles of caregiver stress over time and the subsequent immune response measures. We analyze these associations in 2 ways, yielding similar conclusions.

First, we treat the stress measurements as responses, stratify the children into 2 groups on the basis of IgE level (or Der f 1 SI or Bla g 2 SI levels in separate analyses), and use covariate adjusted non-parametric smoothing to estimate 2 curves of average stress over the child's age for the dichotomous outcomes. Smoothing was done with the linear mixed model formulation of penalized splines, with family-specific random intercepts included in the model to allow for within-family correlation.³³ In this approach differences between the curves at a specific age indicate an association between average stress exposure and different immune responses at that specific age.

The second approach treated the dichotomized immune outcome as the response and used a summary statistic derived from each family's stress curve as a covariate. The time interval for the stress summary statistics was suggested from the nonoverlapping regions in the spline smooth average stress curves for total IgE and the SI. We tested whether the index children from those family's having mean stress levels over specific developmental intervals (on the basis of the

child's age) of greater than the population median stress level over the same age interval expressed higher levels of IgE or demonstrated a higher lymphocyte proliferative response (SI >3). The analyses with dichotomized IgE, Der f 1 SI, and Bla g 2 SI as the outcomes were done by using multiple logistic regressions.

Multiple linear regression was used to examine the relationship between caregiver stress and the cytokine outcomes. Logarithmic transformation of the cytokine outcomes was used to symmetrize the residuals.

RESULTS

Table 1 shows the distribution of sociodemographic factors, maternal asthma, and whether the child had a cold within a week of the blood draw relative to total IgE levels and allergen-specific SIs. Fig 1 contains estimates of the stress curves for the families stratified by IgE level (IgE > 100 IU/mL vs IgE ≤ 100 IU/mL) or Der f 1 SI (SI > 3 vs SI ≤ 3). In these data IgE level (mean ± SD, 40.2 ± 95.2 IU/mL; range, 2-1247 IU/mL) and Der f 1 SI (5.5 ± 6.2; range, 0.4-34.0) showed considerable variability. Total IgE and Der f 1 SI levels were dichotomized into distinct subsets to limit the influence of high leverage

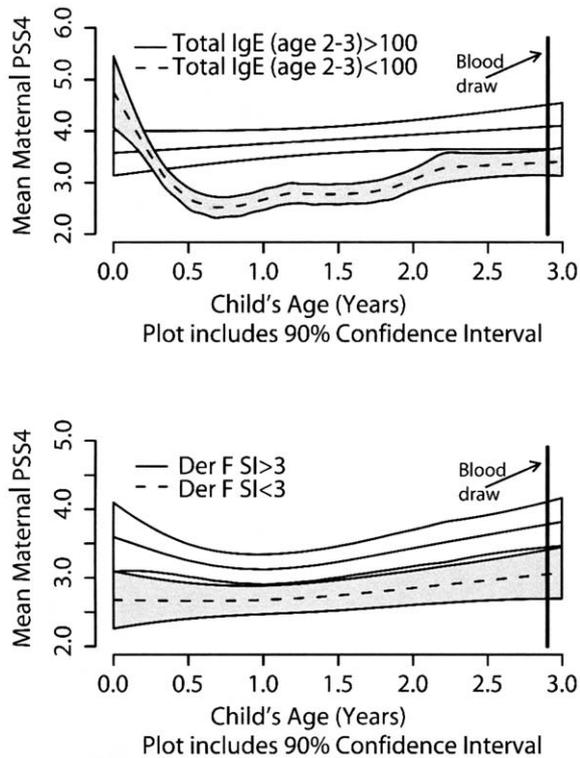


FIG 1. Plots derived from the linear mixed model formulation of penalized splines with family-specific random intercepts included in the model to allow for within-family correlation of repeated stress measurement. Analysis controls for race, active maternal asthma, and cold status. Proliferation was quantified by the SI, which was defined as the mean thymidine incorporation of the stimulated sample divided by that of the unstimulated sample. The SI was considered to be a binary variable indexed as a high (SI >3) or low (SI \leq 3) response.

and outlying observations. From shortly after birth (ie, age 2-3 months) onward, the mean of repeated measures of caregiver stress was persistently higher among children who had a higher Der f 1 SI and among those who expressed higher levels of IgE. Findings were similar for the lymphocyte proliferative response to Bla g 2, with higher-level stress related to a high SI, although the curves overlapped and are not shown.

In the second approach using logistic regression analyses to estimate the relationship between caregiver PSS scores and the probability that the child would have a higher lymphocyte proliferative response to antigen at age 2 to 3 years (SI >3 vs SI \leq 3), higher caregiver stress averaged over time was associated with the higher-level response to Der f 1. In regression analyses predicting total serum IgE levels (IgE >100 IU/mL vs IgE \leq 100 IU/mL), higher mean caregiver stress averaged over time was also associated with higher IgE levels. In the multiple logistic regression analyses predicting IgE, increased stress between ages of 6 and 18 months was associated with an increased probability of a higher total IgE level (>100 IU/mL; odds ratio [OR], 2.03; 95% CI, 1.13-3.63). Increased stress in the child's first 4 months was associated with a Der f 1 SI of greater than 3 (OR, 1.51; 95% CI, 1.00-

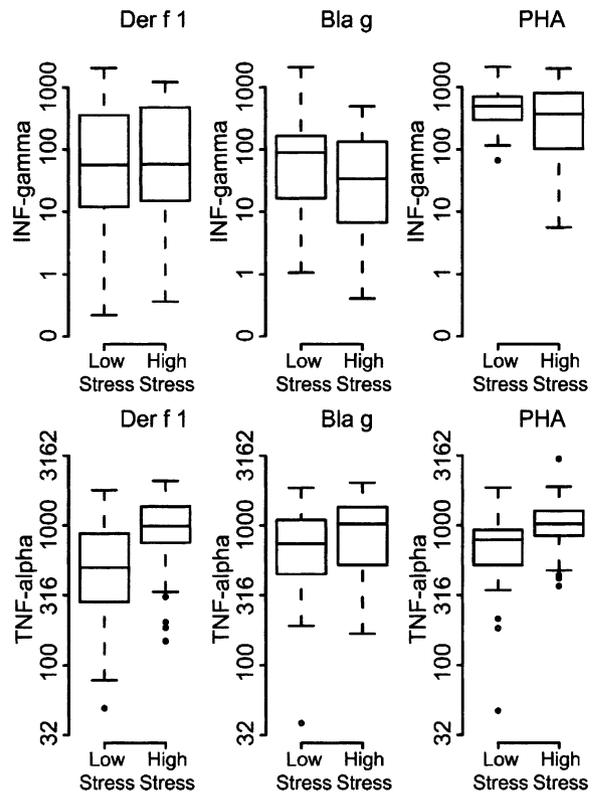


FIG 2. Modified box plots representing the distribution of TNF- α and IFN- γ expression in supernatants taken from allergen-stimulated (dust mite [Der f 1] and cockroach [Bla g]) and mitogen-stimulated (PHA) PBMCs stratified by caregiver stress levels. Stress was categorized as greater than (*High*) or less than (*Low*) the study population median averaged over the first 2 years of the child's life. The upper and lower bounds on the boxes represent the first and third quartiles (25th and 75th percentile of the sample, respectively), the line within the box denotes the median, the whiskers represent values within $1.5 \times$ quartile 3 (*top*) or quartile 1 (*bottom*), and the dots show values outlying this range. The y-axis represents actual values that are log spaced.

2.29) and nominally associated with a Bla g 2 SI of greater than 3 (OR, 1.13; 95% CI, 0.72-1.78). These analyses are adjusted for age, maternal asthma, sex, cold status, household allergen exposure at age 2 to 3 months, and race-ethnicity.

Finally, we examined the relationship between caregiver stress and cytokine production. Fig 2 shows the distribution of IFN- γ and TNF- α expression from allergen- and mitogen-stimulated PBMCs. Although there is considerable spread within groups, TNF- α levels tend to be higher and IFN- γ levels tend to be lower in children whose caregivers reported more stress. In multiple linear regressions predicting the logarithm of the cytokines (Table 2), a higher TNF- α level was significantly associated with increased caregiver stress (mean stress of greater than the study population median averaged over the first 2 years of the child's life) for allergen-specific and mitogen-induced lymphocyte proliferation. In addition, higher stress predicted lower levels of IFN- γ in the mitogen-induced response. Associations between higher stress and lower

TABLE II. Risk ratio of increased early childhood stress* predicting cytokine production†

Lymphocyte stimulation factor	IFN- γ : Risk ratio‡ high vs low stress (95% CI)	TNF- α : Risk ratio high vs low stress (95% CI)	IL-10: Risk ratio high vs low stress (95% CI)	IL-13: Risk ratio high vs low stress (95% CI)
Der f 1§	0.96 (0.51-1.80)	1.82# (1.28-2.59)	1.03 (0.71-1.50)	0.75 (0.47-1.20)
Bla g	0.86 (0.56-1.32)	1.55# (1.18-2.04)	1.00 (0.74-1.34)	0.97 (0.62-1.52)
PHA¶	0.72¶ (0.53-0.98)	1.75¶ (1.09-2.80)	1.07 (0.80-1.44)	0.84 (0.64-1.11)

*Each analysis compares the caregiver's mean stress during the child's first 2 years, being greater than that of the study population median caregiver stress for the same time period as the referent group (ie, those greater than the median are the high-stress group).

†All models (one for each 4 cytokines for Der f 1, Bla g 1 or 2, and PHA) are adjusted for age, sex, race, income, maternal active asthma, household maximum allergen exposure, and report of child having a cold in the week before the blood draw collected for lymphocyte proliferation.

‡The risk ratio is defined with low stress as the referent group. That is, a risk ratio of 2 indicates that increased early childhood caregiver stress is associated with a 2-fold increase in a particular cytokine expression among the index children on average.

§*Dermatophagoides farinae* (Der f 1), dust mite antigen.

||Cockroach (Bla g 1 or Bla g 2).

¶ $P < .05$.

$P < .01$.

IFN- γ levels were suggested for allergen-induced responses, but these did not reach statistical significance. Models were adjusted for race, age at blood draw, sex, household allergen exposure, maternal asthma, and whether the child had a cold in the week preceding the blood draw.

DISCUSSION

These data demonstrate that higher early-life chronic caregiver stress was associated with increased total IgE expression and an enhanced allergen-specific proliferative response at age 2 to 3 years. Moreover, higher-level stress was associated with increased TNF- α levels by stimulated PBMCs and reduced IFN- γ levels. Exposure to stress in early development might result in functional changes in immune reactivity in susceptible children, potentiating the inflammatory response.

Serial measurement of IgE is an indicator of whether an atopic phenotype is developing in a child.⁸ Increased IgE levels might occur during the period of T-cell maturation, when an individual's phenotype is being determined not only by exposure to allergens but also by a variety of environmental influences. These data suggest that early life stress might be an additional factor influencing this process, given the significant relationship between higher mean caregiver stress and high IgE levels (>100 IU/mL). Given the small number of subjects in the high-IgE group, reproduction of these findings in other studies with larger sample size would increase certainty about their generalizability.

These results corroborate others, suggesting that chronic stress might influence cytokine production, although data in early childhood are sparse. The association between stress and decreased ability of peripheral blood lymphocytes to synthesize IFN- γ when stimulated *in vitro* has been reported.³⁴ The suggestion that IFN- γ production is reduced in the context of higher stress is interesting in light of prospective seroepidemiologic evidence demon-

strating that decreased IFN- γ and IL-2 production by stimulated lymphocytes in infancy predicts later development of atopy.⁸ IFN- γ levels tended to be lower in children whose caregivers reported more stress for both mitogen-induced and allergen-specific stimulation. Other analyses of these data have demonstrated decreased expression of IFN- γ for both Der f 1 and Bla g 2 stimulation among children with both atopic disease (ie, eczema) and wheeze when compared with those with no atopic disease and no history of wheeze.³⁵ Sample size did not allow further stratification by these disease categories to test whether the relationship between increased caregiver stress and reduced IFN- γ production might also be stronger among children expressing atopy.

Catecholamines, glucocorticoids, and proinflammatory cytokines (including TNF- α) are considered the principal messengers between the nervous system and immune system in the stress response.¹³ Increased TNF- α levels can activate the HPA axis and have been associated with increased cortisol levels.³⁶ Cortisol has been shown to shift T cells toward a T_H2 phenotype.³⁷ Others have demonstrated a relationship between stressor-induced cortisol and increased IgE production.³⁸ In chronic stress more persistently increased TNF- α levels might result in dysregulation of the HPA axis,³⁹ which might in turn play a role in the development of childhood asthma.¹³

Notably, the most consistent finding in these analyses suggests that chronic stress enhances the production of TNF- α . This is interesting in light of evidence that TNF- α , produced by both lymphocytes and macrophages, is involved in the atopic immune response.^{5,6} TNF- α also participates in the recruitment and activation of many inflammatory cell types and stimulates the synthesis of nitric oxide and other inflammatory mediators that drive chronic delayed-type inflammatory responses.^{40,41}

Although we identify associations between stress and cytokine expression (eg, increased TNF- α) in these epidemiologic data, at this stage it is not clear whether stress preceded the increase of TNF- α production or whether TNF- α is a mediator of the effect of stress on allergic or airway disease expression. Future epidemiologic studies

are needed to explore this further. Similarly, complementary animal studies informed through these epidemiologic findings might explore suggested mechanisms, as discussed below.

Although we had only a single measure of the immune response at age 2 to 3 years, it is interesting to speculate on the potential influence of stress-induced increased TNF- α levels during earlier development. That is, during early maturation of the infant's immune system, TNF- α might be produced by antigen-presenting cells, such as monocytes-macrophages, dendritic cells^{4,42} and mast cells,⁴³ playing an important role in the interactions between innate and adaptive immunity. Innate immune cytokines, such as TNF- α , are likely involved in priming the adaptive immune-humoral responses, but it is not known whether, in some children, this priming results in an increased predisposition to allergy and allergic disease. Notably, recent evidence has linked TNF- α to the development of allergic rhinitis in mice.⁴⁴ The influence of stress on the innate immune response warrants further study.

Moreover, many effects of TNF- α ⁴⁵ are mediated by the induction of a cellular state consistent with oxidative stress.⁴⁶ Oxidative stress has been implicated in asthma and allergy.^{47,48} Overlapping research suggests that psychological stress might augment oxidative toxicity. Emotionally stressed rats have increased levels of 8-deoxy-hydroxy-guanosine (8-OhdG), a biomarker of oxidative stress.⁴⁹ Similarly, staying awake all night increased the levels of thiobarbituric acid-reactive substances in human subjects.⁵⁰ Irie et al⁵¹ used classical conditioning to illustrate the role of chronic stress and oxidative damage.

In summary, chronic stress in early childhood might influence the immune response manifested by an enhanced allergen-specific response, increased production of TNF- α , reduced production of IFN- γ , and increased serum IgE expression. Although the T_H1/T_H2 paradigm remains an important functional dichotomy to consider when interpreting quantitative differences in cytokine expression in response to environmental stimuli like stress, examination of other mechanisms (eg, oxidative stress pathways or innate immune factors) or a broader range of cytokines produced by cells both within and outside the immune system might better delineate the true complexity of the underlying mechanisms linking stress to allergic sensitization and asthma. Moreover, research linking caregiver stress to atopic sensitization might change how we think about preventative strategies. These findings suggest that future interventions should include clinical approaches and, more broadly, public policies designed to strengthen families and reduce parenting stress.

REFERENCES

- Donovan CE, Finn PW. Immune mechanisms of childhood asthma. *Thorax* 1999;54:938-46.
- Romagnani S. Induction of TH1 and TH2 responses: a key role for the 'natural' immune response? *Immunol Today* 1992;13:379-81.
- Romagnani S. Human TH1 and TH2 subsets: doubt no more. *Immunol Today* 1991;12:256-7.
- Mosman TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996;17:138-46.
- Kim MH, Agrawal DK. Effect of interleukin-1 beta and tumor necrosis factor-alpha on the expression of G-proteins in CD4+ T-cells of atopic asthmatic subjects. *J Asthma* 2002;39:441-8.
- Halasz A, Cserhati E, Magyar R, Kovacs M, Cseh K. Role of TNF-alpha and its 55 and 75 kDa receptors in bronchial hyperreactivity. *Respir Med* 2002;96:262-7.
- Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;92:313-24.
- Burrows B, Martinez FD, Cline MG, Lebowitz MD. The relationship between parental and children's serum IgE and asthma. *Am J Respir Crit Care Med* 1995;152:1497-500.
- Holt PG, Yabuhara A, Prescott SL, Venaille TJ, Macaubas C, Holt BJ, et al. Allergen recognition in the origin of asthma. In: Holgate S, editor. *The rising trends in asthma*. Chichester (UK): John Wiley & Sons; 1997. p. 35-55.
- Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730-7.
- Piccinni MP, Mecacci F, Sampognaro S, Manetti R, Parronchi P, Maggi E, et al. Aeroallergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993;102:301-3.
- Finn PW, Boudreau JO, He H, Chapman MD, Carey V, Burge HA, et al. Children at risk for asthma: home allergen levels, lymphocyte proliferation, and wheeze. *J Allergy Clin Immunol* 2000;105:933-42.
- Wright R, Rodriguez M, Cohen S. Review of psychosocial stress and asthma: an integrated biopsychosocial approach. *Thorax* 1998;53:1066-74.
- Peebles RS, Togias A, Bickel C, Diemer FB, Hubbard WC, Schleimer RP. Endogenous glucocorticoids and antigen-induced acute and late phase pulmonary responses. *Clin Exp Allergy* 2000;30:1257-65.
- Patterson JM. Conceptualizing family adaptation to stress. In: Tanner JL, editor. *Report of the Twenty-Fifth Roundtable on Critical Approaches to Common Pediatric Problems*. Columbus (OH): Ross Products Division, Abbott Laboratories; 1995. p. 11.
- Vallee M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J Neurosci* 1997;17:626-36.
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis DJ, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal response to stress. *Science* 1997;277:1659-62.
- Ramsey DS, Lewis D. Developmental change in infant cortisol and behavioral response to inoculation. *Child Dev* 1994;65:1491-502.
- Gold DR, Burge HA, Carey V, Milton DK, Platts-Mills T, Weiss ST. Predictors of repeated wheeze in the first year of life: the relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. *Am J Respir Crit Care Med* 1999;160:227-36.
- Chapman M, Aalberse RC, Brown MJ, Platts-Mills T. Monoclonal antibodies to the major feline allergen Fel d I, II: single step affinity purification of Fel d I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess Fel d I exposure. *J Immunol* 1988;140:812-8.
- Cohen S, Williamson G. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, editors. *The social psychology of health*. Newbury Park (CA): Sage; 1988. p. 31-67.
- Cronbach L. Coefficient alpha and the internal structure of tests. *Psychometrika* 1951;16:297-334.
- Lazarus RS, Folkman S. *Stress, appraisal, and coping*. New York: Springer; 1984.
- Cohen S, Kessler RC, Underwood Gordon L. *Measuring stress: a guide for health and social scientists*. New York: Oxford University Press; 1995.

25. Ng DM, Jeffery RW. Relationships between perceived stress and health behaviors in a sample of working adults. *Health Psychol* 2003;22:638-42.
26. Cohen S, Lichtenstein E. Perceived stress, quitting smoking, and smoking relapse. *Health Psychol* 1990;9:466-78.
27. Cohen S, Doyle WJ, Skoner DP. Psychological stress, cytokine production, and severity of upper respiratory illness. *Psychosom Med* 1999;61:175-80.
28. Cohen S, Tyrrell DAJ, Smith AP. Psychological stress in humans and susceptibility to the common cold. *N Engl J Med* 1991;325:606-12.
29. Wright RJ, Cohen S, Carey V, Weiss ST, Gold DR. Parental stress as a predictor of wheezing in infancy: a prospective birth-cohort study. *Am J Respir Crit Care Med* 2002;165:358-65.
30. Lewis S, Weiss ST, Platts-Mills T, Syring M, Gold DR. Association of specific allergen sensitization with socioeconomic factors and allergic disease in a population of Boston women. *J Allergy Clin Immunol* 2001;107:615-22.
31. Busse WW, Rosenwasser LJ. Mechanisms of asthma. *J Allergy Clin Immunol* 2003;111(suppl 3):S799-804.
32. Lara-Marquez ML, Deykin A, Krinzman S, Listman J, Israel E, He H, et al. Analysis of T-cell activation after bronchial allergen challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1998;101:699-708.
33. Ruppert D, Wand M, Carroll R. *Semiparametric Regression*. New York: Cambridge University Press; 2003.
34. Manuck SB, Cohen S, Rabin BS, Muldoon MF, Bachen EA. Individual differences in cellular immune response to stress. *Psychol Sci* 1991;2:111-5.
35. Contreras JP, Ly NR, Gold DR, Hongzhen H, Wand M, Weiss ST, et al. Allergen induced cytokine production, atopic disease, IgE and wheeze in children. *J Allergy Clin Immunol* 2003;112:1072-7.
36. Fukata J, Imura H, Nakao K. Cytokines as mediators in the regulation of the hypothalamic-pituitary-adrenocortical function. *J Endocrinol Invest* 1993;16:141-55.
37. Ramirez F, Fowell DJ, Puklavec M, Simmonds S, Mason D. Glucocorticoids promote a Th2 cytokine response by CD4+ T cells in vitro. *J Immunol* 1996;156:2406-12.
38. Wu CY, Sarfati M, Heusser C, Fournier S, Rubio-Trujillo M, Peleman R, et al. Glucocorticoids increase the synthesis of immunoglobulin E by interleukin-4 stimulated human lymphocytes. *J Clin Invest* 1991;87:870-7.
39. Chrousos GP. Stress, chronic inflammation, and emotional and physical well-being: concurrent effects and chronic sequelae. *J Allergy Clin Immunol* 2000;106(suppl):S275-91.
40. Drost EM, MacNee W. Potential role of IL-8, platelet-activating factor and TNF-alpha in the sequestration of neutrophils in the lung: effects on neutrophil deformability, adhesion receptor expression, and chemotaxis. *European J Immunol* 2002;32:393-403.
41. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 1999;399:601-5.
42. Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. *Science* 1996;272:50-3.
43. Okumura S, Kashiwakura J, Tomita H, Matsumoto K, Natkajima T, Saito H, et al. Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcepsilonRI. *Blood* 2003;102:547-54.
44. Iwasaki M, Saito K, Takemura M, Sekikawa K, Fujii H, Yamada Y, et al. TNF-alpha contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol* 2003;112:134-40.
45. Bachen EA, Manuck SB, Cohen S, Muldoon MF, Raible R, Herbert TB, et al. Adrenergic blockade ameliorates cellular immune responses to mental stress in humans. *Psychosom Med* 1995;57:366-72.
46. Glosli H, Tronstad KJ, Wergedal H, Muller F, Svardal A, Aukrust P, et al. Human TNF-alpha in transgenic mice induced differential changes in redox status and glutathione-regulating enzymes. *FASEB* 2002;16:1450-2.
47. Koyama H, Geddes DM. Genes, oxidative stress, and the risk of chronic obstructive pulmonary disease. *Thorax* 2000;55(suppl):S51-3.
48. Fryer AA, Bianco A, Hepple M, Jones PW, Strange RC, Spiteri MA. Polymorphism at the glutathione S-transferase GSTP1 locus. A new marker for bronchial hyperresponsiveness and asthma. *Am J Respir Crit Care Med* 2000;161:1437-42.
49. Adachi S, Kawamura K, Takemoto K. Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress. *Cancer Res* 1993;53:4153-5.
50. Kosugi H, Enomoto H, Ishizuka Y, Kikugawa K. Variations in the level of urinary thiobarbituric acid reactant in healthy humans under different physiological conditions. *Biol Pharm Bull* 1994;17:1645-50.
51. Irie M, Asami S, Nagata S, Miyata M, Kasai H. Classical conditioning of oxidative DNA damage in rats. *Neurosci Lett* 2000;288:13-6.