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Inflammatory Proteins Predict Change in Depressive Symptoms in Male and Female Adolescents

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Abstract
Inflammation has been implicated in depressive symptoms, but few studies use longitudinal designs with adolescents. Furthermore, the extant literature has yielded inconsistent results. Blood was collected from a community sample of 201 adolescents (109 female, age range = 12.3–20.0 years) and analyzed for inflammatory proteins. Up to five follow-up assessments of depressive symptoms were conducted. Multilevel modeling indicated that high C-reactive protein (CRP) but no other proinflammatory markers predicted depressive symptom increases. Three-way interactions between different inflammatory biomarkers, sex, and months to follow-up predicted change in depressive symptoms. Higher interleukin-6 predicted increased depressive symptoms at 13 to 31 months after baseline assessment of depression and inflammation for females. Higher tumor necrosis factor-α predicted increased depressive symptoms at < 1 month after baseline for males and 13 to 31 months after baseline for females. Higher interleukin-8 in males predicted lower depressive symptoms at 31 months after baseline. Exploratory post hoc analyses were used to examine these predictive associations for specific subsets of depressive symptoms. These findings are the first to support the predictive association of elevated CRP for depressive symptoms in a community adolescent sample and serve as preliminary evidence that the relationship between cytokines and later depressive symptoms differs by sex, time to follow-up, and the specific biomarker.

Keywords
depression, longitudinal methods, risk factors, sex differences, adolescent development

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Over the past 2 decades, there has been increasing interest in the role of inflammation and the immune system in the pathophysiology of depression. This newer perspective is due in part to research investigating the increased risk for depression among individuals with inflammation-related conditions, including autoimmune disorders (Calder, 2006; Goodwin, Fergusson, & Horwood, 2004; Ohayon & Schatzberg, 2003; Pan et al., 2012; Whooley, 2006). Further evidence has come from studies documenting higher levels of several proinflammatory cytokines—for example, interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α)—and acute phase reactants such as C-reactive protein (CRP) in individuals with depression compared with nondepressed controls (Dhabhar et al., 2009; Dowlati et al., 2010; Howren, Lamkin, & Suls, 2009). Proinflammatory cytokines are signaling proteins that are released primarily to stimulate cellular functions and are upregulated during infection or after trauma, but they also serve to communicate with the brain about the body's healthy or challenged status (Janeway, 1989).

An increase in proinflammatory cytokines was first shown to modulate behavior in animals (Hart, 1988). Specifically, administering cytokines or mimicking an infection with bacterial endotoxin was found to induce

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depression-related behavior, including malaise, fatigue, anorexia, and a reduced interest in rewarding activities (i.e., anhedonia; Dantzer & Kelley, 2007; Watkins & Maier, 1999). The overlap with several diagnostic criteria for depressive disorders led a number of researchers to investigate whether cytokine activity was dysregulated in depressed individuals and determine if immunomodulatory clinical treatments, such as interferon therapy, would elicit similar symptoms (American Psychiatric Association, 2013; Capuron & Miller, 2004). It is believed that these somatic and psychological changes, often described as sickness behavior, are initially adaptive and enable a diversion of energy resources for host protection but ultimately become maladaptive when sustained (Antoni et al., 2012; Irwin & Cole, 2011). Moreover, when recurrent, especially when evoked by psychological challenges and threats rather than infectious pathogens, the chronic subclinical activation can become a risk/maintenance factor for disease such as asthma, diabetes, and Alzheimer's disease (Couzin-Frankel, 2010). In addition, this inflammatory activity can have collateral effects on endocrine regulation, especially for the hypothalamic-pituitary-adrenal axis, resulting in a downregulation of glucocorticoid receptors and reduced hormonal feedback on inflammatory pathways. This proinflammatory phenotype has been hypothesized to be associated with and accentuate depressive symptoms (A. H. Miller, Maletic, & Raison, 2009; G. E. Miller, Cohen, & Ritchey, 2002). Although inflammation has been consistently associated with depression and depressive symptoms (Howren et al., 2009; A. H. Miller et al., 2009), the vast majority of the extant literature on this topic utilizes cross-sectional studies. The utilization of prospective study designs is critical to determine potential causality and directionality of the relationship between inflammation and depression.

Considering longitudinal studies, elevated levels of CRP and IL-6 were found to be predictive of future depressive symptoms at a 12-year follow-up in middle-aged adults (Gimeno et al., 2009). Zalli, Jovanova, Hoogendijk, Tiemeier, and Carvalho (2016) also demonstrated that higher baseline levels of IL-6 and CRP were associated with persistence of depressive symptoms at a 5-year follow-up in a geriatric Dutch sample (mean age = 73 years). However, these predictive relationships are not always overtly evident. Neither van den Biggelaar et al. (2007) nor Stewart, Rand, Muldoon, and Kamarck (2009) found an association between IL-6 and future depressive symptoms at 6- and 5-year follow-ups, respectively, although van den Biggelaar et al. found that CRP levels at baseline predicted future depression. Notably, there is considerable heterogeneity among the results of extant studies evaluating the predictive association of inflammatory activity for future depressive symptoms. For example, there is substantial variation in the length of time to follow-up, a factor that might contribute to differences in results. To date, there have been no studies investigating how long lasting the effects of inflammation on mental health might be; thus, it may be that certain biomarkers are associated with behavioral outcomes for different lengths of time. Alternatively, it may be that some inflammatory biomarkers influence behavior through the concept of allostatic loading and longer periods of time are necessary to see their effects on mental health. It is also unclear whether these findings would extend to adolescents, especially because some of the biomarkers, such as CRP, are typically lower in younger individuals and others, like IL-6, often reflect a significant contribution of the weight gain and obesity that emerge later in adulthood.

Association Between Inflammation and Adolescent Depression

There is a dearth of prospective studies investigating the role of inflammation in the onset of depressive symptoms among adolescents, a critical period for the development of depressive symptoms (Hankin et al., 1998). For some adolescents, these depressive symptoms progress to clinical diagnoses, highlighting the importance of studying risk factors for increases in depressive symptoms during this developmental period (van Lang, Ferdinand, & Verhulst, 2007). Research on the antecedents of depression in this time period is particularly important because adolescent-onset depression is associated with significant functional impairment, increased suicide risk, and a chronic course of depression throughout the life span (Balázs et al., 2013; Gilman, Kawachi, Fitzmaurice, & Buka, 2003). In addition, studying inflammation as a predictor of subsequent increases in depression during adolescence has additional design advantages in that adolescents, in general, may possess fewer confounders of inflammation (e.g., sedentary lifestyle, obesity, and substance use) than adults (Harris, Gordon-Larsen, Chantala, & Udry, 2006). Moreover, because CRP and proinflammatory cytokine levels are typically lower in adolescence than later in adulthood, the association between inflammation and depressive symptoms also may differ in adolescents than in adults.

In an adolescent sample, Copeland, Shanahan, Worthman, Angold, and Costello (2012) did not find a significant relationship between CRP and later depression with follow-ups at 1 to 3 years; however, they did demonstrate that cumulative episodes of depression were prospectively associated with higher levels of CRP. G. E. Miller and Cole (2012) reported a bidirectional relationship between CRP and depressive symptoms at

6-month follow-ups but only in female adolescents with a history of childhood adversity. Notably, this finding highlights the potential for the relationship between inflammation and depression to be stronger for females than males. Duivis et al. (2015) found that persistent moderate or high depressive symptoms were associated with higher subsequent CRP levels across three biennial assessments. These findings supporting a bidirectional relationship between inflammation and depression implicate inflammation as a mechanism underlying depression in adolescents, but further study is clearly needed. Additionally, the findings highlight the importance of utilizing longitudinal data sets with repeated measures of both inflammation and depression to be able to test bidirectionality of results. However, prospective designs in adolescent samples that examine inflammation as a predictor of subsequent depressive symptoms at multiple time points, such as the current study, also are valuable in testing whether elevated inflammation is a marker of increasing depressive symptoms over time.

Sex/Gender Differences in Inflammation and Depression

There are established sex/gender-based differences in both inflammation and the development of depression during adolescence. The prevalence of depression shows strong gender differences in adolescence (Costello, Erkanli, & Angold, 2006; Hankin et al., 1998). Additionally, depressive symptoms have been demonstrated to increase more for girls than boys during adolescence (Garber, Keiley, & Martin, 2002). In addition, studies of adults have found sex differences in the concentrations of proinflammatory biomarkers comparing premenopausal adult women and adult men (Cartier et al., 2009). But we still know relatively little about sex-related differences in normative CRP and cytokine levels during adolescence, although one study found sex differences in the concentrations of specific cytokines in adolescents (Pallavi et al., 2015). Further, similar life stressors (e.g., childhood victimization) have been demonstrated to affect the inflammatory profiles of male and female adolescents differently (Baldwin et al., 2018). Taken together with the evidence reviewed by Bangasser, Eck, and Sanchez (2019) that there are sex differences in biological stress reactivity that predispose women to psychiatric disorders featuring hyperarousal (e.g., depression) and men to psychopathology involving attentional abnormalities (e.g., attention-deficit hyperactivity disorder), it is plausible to hypothesize that the role that inflammation plays in the etiology of depression differs for males and females. Additionally, as described previously, at least one published study has found significant associations between inflammation and depression exclusively in females (G. E. Miller & Cole, 2012). Thus, inflammation could theoretically be a more salient biological mechanism underlying depression for females compared with males.

The Current Study

Our study prospectively investigated the value of five inflammatory biomarkers (CRP, IL-6, IL-8, IL-10, and TNF-α) for predicting the development of depressive symptoms in an ethnically diverse community sample of male and female adolescents. This panel of biomarkers was chosen because of their frequent use in depression research, maximizing the utility of results from this study to inform the interpretation of results from past studies and design of future studies. Investigating this question in the context of a community sample is important because much of the extant literature focuses on clinical or at-risk populations (e.g., Dhabhar et al., 2009; Dowlati et al., 2010; Howren et al., 2009; G. E. Miller & Cole, 2012). Additionally, we investigated the potential for sex (we include biological sex rather than gender because inflammation is a biological variable) and the time interval between the assessment of inflammation and depressive symptoms to moderate this relationship. This analytical approach allows for a novel investigation of the methodological consideration of time to follow-up while also testing sex differences in the risk inflammation confers for the development of depressive symptoms. The a priori hypothesis was that higher CRP and proinflammatory cytokine levels would predict increases in depressive symptoms and that higher IL-10, an anti-inflammatory cytokine, would predict decreases in depressive symptoms. Given the known sex differences in the prevalence of depression in adolescents and the tendency for inflammatory conditions to be more common in women, we hypothesized that the influence of inflammatory activity on the development of depressive symptoms would be stronger in females. No directional hypotheses were made in regard to how time to follow-up might influence the relationship between inflammation and the development of depressive symptoms because previous research has found results with follow-up periods ranging from 6 months (G. E. Miller & Cole, 2012) to 12 years (Gimeno et al., 2009).

Method and Materials

Participants

Participants were drawn from the Adolescent Cognition and Emotion (ACE) project at Temple University. A community sample of adolescents aged 12 to 13 and their

Table 1. Descriptive Statistics

Statistic	Mean	Proportion	Standard Deviation
Time 1 age	16.84		1.16
Family income[a]: ≤ $14,999–$29,999		29	
Family income[a]: $30,000–$59,999		36	
Family income[a]: $60,000–$89,999		16	
Family income[a]: ≥ $90,000		18	
Female		54	
White		38	
African American		58	
Biracial		4	
C-reactive protein (mg/L)	3.97		9.86
Interleukin-6 (mg/L)	0.60		0.67
Interleukin-8 (mg/L)	3.27		2.11
Interleukin-10 (mg/L)	0.39		0.73
Tumor necrosis factor-α (mg/L)	1.64		0.78
Time 1 depression (CDI)	6.82		5.97
Time 2 depression (CDI)	6.91		6.57
Time 3 depression (CDI)	6.74		7.01
Time 4 depression (CDI)	5.94		6.39
Time 5 depression (CDI)	6.88		7.35
Time 6 depression (CDI)	5.28		5.05

Note: CDI = Children's Depression Inventory.
[a]Sum of proportions for family income does not equal 100 because of rounding.

mothers or primary female caregivers were recruited from the greater Philadelphia area. Recruitment for this study involved a combination of mailings and follow-up calls to families with children attending Philadelphia public and private middle schools (68% of the total sample) and advertisement in local newspapers (32% of the sample). Inclusion criteria included sufficient competence with the English language to complete the assessments. Additionally, participants had to identify as either White, African American, or biracial because the investigation of differences in the etiology of depression comparing these three demographic groups was one of the aims of Project ACE. All demographic information was self-reported during the first visit of the study. Exclusion criteria included a history of severe psychiatric illness or developmental disorders (see Alloy et al., 2012, for further information). Informed written consent was obtained from mothers and written assent from adolescents prior to data collection. The Temple University Institutional Review Board approved the protocol (IRB protocol No. 6844).

The current sample consisted of a subsample of 201 adolescents who volunteered to participate in an optional blood draw after the main study had been ongoing. The data set for this study included up to five follow-up depressive symptom assessments (some participants had more follow-ups than others because the parent study is currently ongoing). At the time of these analyses, there were 582 observations across 201 participants (mean age at blood draw = 16.8 years; *SD* = 1.2 years; range = 12.3–20.0 years). An attrition analysis was conducted to determine whether any demographic variables were associated with number of follow-ups after the blood draw. Results indicated that higher age at blood draw was associated with fewer follow-ups ($t = -3.308$, $p < .01$). No other variables were significantly associated with number of follow-ups (all *p*s >.05). The final sample was 54% female, 38% White, 58% African American, and 4% biracial (see Table 1 for descriptive statistics and Table S1 in the Supplemental Material available online for a correlation matrix of study variables). Throughout the duration of the study, 23% of participants reported depressive symptom scores suggesting at least mild depression (Bang, Park, & Kim, 2015).

Measures

Depressive symptoms. Symptoms of depression were measured using the Children's Depression Inventory (CDI; Kovacs, 1985). It consists of 27 items reflecting affective, behavioral, and cognitive symptoms of depression. Items are rated on a scale from 0 to 2, and total scores range from 0 to 54. The CDI has been demonstrated to be a reliable and valid measure of depressive symptoms in youth samples (Klein, Dougherty, & Olino,

2005). The CDI was administered both at the Time 1 (T1) blood draw and at up to five follow-up assessments. Internal consistency in this sample was α = .84 at the first study visit. The total CDI score was used as the dependent variable for the main hypothesis-testing analyses. For exploratory, post hoc analyses, five depressive symptom subscales were used, in accordance with a meta-analytic factor structure of the CDI in adolescents (Huang & Dong, 2014). The five subscales were somatic concerns (nine items; α = .60), externalizing (six items; α = .58), negative self-concept (seven items; α = .60), lack of personal and social interest (five items; α = .66), and dysphoric mood (four items; α = .65).

Inflammation. Blood was obtained via antecubital venipuncture by a certified phlebotomist into a 10 ml vacutainer designed for freezing plasma separated from the cells within the vial (BD Hemogard with K2 EDTA). Vacutainers were stored in an ultracold freezer at −80°C and later thawed on the day of assay. Collection time for the blood draw and participants' body mass index (BMI; based on direct measurement of height and weight) were recorded.

Four cytokines were quantified by multi-cytokine array (IL-6, IL-8, IL-10, and TNF-α), and high-sensitivity CRP was determined in a singleplex assay using an electrochemiluminescence platform and a QuickPlex SQ 120 imager for analyte detection (Meso Scale Discovery, Gaithersburg, MD). Each specimen was assayed in duplicate. The intraassay coefficients of variation were 5.36 and 2.29 for the cytokines and CRP, respectively. Plasma was diluted 1:2 for the cytokine assay and 1:1,000 for the CRP assay. Values were calculated with respect to a standard curve generated from 7 calibrators with known concentrations. The lower limit of detection (LLOD) for the cytokines was 0.1 pg/ml, with a large dynamic range up to 2,000 pg/ml. CRP is present in blood at higher concentrations, and thus, plasma was diluted to correspond to the standard curve. The LLOD for CRP was 0.1 mg/l. Values below the LLOD were set at the LLOD. Values were converted to milligrams per liter to be consistent with the clinical literature (Breen et al., 2011; Dabitao, Margolick, Lopez, & Bream, 2011).

Procedure

At T1, adolescents completed an optional blood draw and the CDI. Ideally, adolescents were scheduled for regular 6-month follow-up visits, with every other follow-up involving two sessions a month apart. However, the timing of participants' follow-ups did not always conform to the planned schedule; thus, the time interval between the T1 blood draw and each follow-up varied across participants. The CDI was administered at each follow-up. All 201 participants had at least one follow-up (months after T1 *M* = 6.5 months, *SD* = 6.3 months), 147 had a second follow-up (months after T1 *M* = 13.2 months, *SD* = 4.7 months), 114 had a third (months after T1 *M* = 16.7 months, *SD* = 4.6 months), 76 had a fourth (months after T1 *M* = 20.5 months, *SD* = 4.0 months), and 44 had a fifth (months after T1 *M* = 24.1 months, *SD* = 3.2 months).

Data analysis plan

All descriptive statistics, correlations, and preliminary analyses were conducted in SPSS (IBM Corp, 2016). Multilevel models were estimated using packages lmer4 (Bates, Maechler, Bolker, & Walker, 2015) and lmerTest (Kuznetsova, Brockhoff, & RHD, 2017) in R x64 3.3.2 (R Core Team, 2013). In addition to using inflammation and sex as person-level predictors, months between blood draw and follow-up was used as an observation-level predictor. Given this study's longitudinal design, months to follow-up was not centered or aggregated. Consequently, months since blood draw estimates a combination of within- and between-subject effects. Additionally, CRP, IL-6, IL-8, TNF-α, and IL-10 were right-skewed (skewness statistics/kurtosis = 5.18, 3.77, 6.52, 6.85, 3.53, respectively); thus, a log transformation (Log[10 × value]) was applied to their raw values, which resulted in skewness statistics that did not violate assumptions of normality (skewness statistics posttransformation = 0.24, 0.72, 1.63, 1.99, 0.43, respectively). To predict to prospective changes in depressive symptoms, the outcome variable was change in CDI score between T1 and follow-up. Multilevel modeling was chosen over traditional regression techniques because the data were clustered within individuals, which would result in greater probability of Type I error and less efficient estimates of coefficients for regression compared with multilevel modeling.

To test the main effects of inflammation on changes in depressive symptoms over time, five identical multilevel models were conducted, each with one of the five inflammatory proteins included (log CRP, log IL-6, log IL-8, log IL-10, and log TNF-α). Models were estimated using restricted maximum likelihood. Each model accounted for the effects of being female, African American, biracial; age at Time 1; parent-report family income; and BMI measured on the day of the blood draw. Additionally, the interaction between each of these variables and months to follow-up was included to account for the within-person effects of these variables. To test the interaction between inflammation, sex, and months to follow-up, each of these five models was rerun with the addition of terms for the interaction between T1 inflammation and sex as well as the

three-way interactions between T1 inflammation, sex, and months to follow-up. Models with significant interactions were probed for each sex and at the minimum value and each quartile of months to follow-up. For post hoc, exploratory analyses, the aforementioned statistical procedures were repeated predicting to each of the five CDI subscales.

Results

Preliminary analyses

Descriptive statistics and bivariate correlations for the main study variables, including untransformed inflammation variables, can be found in Table 1 and Table S1, respectively. Additionally, the descriptive statistics for the biomarker variables prior to log-transformation and baseline depressive symptoms for males and females is in Table S2 in the Supplemental Material. We also conducted independent sample *t* tests to test whether the levels of log-transformed inflammation variables or baseline depressive symptoms differed significantly for males and females. Results indicated that both log CRP and log IL-6 were higher in females than in males, $t(199) = -2.456$, $p = .015$; $t(199) = -2.559$, $p = .011$, respectively (see Table S2). These results were not robust to Bonferroni corrections (adjusted p value = .01), although the fact that Bonferroni corrections tend to be overly conservative should be taken into consideration (Bland & Altman, 1995). Levels of log IL-8, log IL-10, and log TNF-α did not differ by sex ($p = .531$, $p = .506$, $p = .938$, respectively). Females also had more depressive symptoms at baseline ($p = .016$). Additionally, five separate linear regressions predicting to total depressive symptoms at baseline from each of the five biomarkers were conducted. Similar to the multilevel models, these analyses controlled for the effects of being female, African American, biracial; age at T1; parent-report family income; and BMI measured on the day of the blood draw. None of the five biomarkers were significantly associated with total depressive symptoms at T1 (log CRP $p = .110$, log IL-6 $p = .083$, log IL-8 $p = .416$, log IL-10 $p = .978$, log TNF-α $p = .119$).

Primary analyses

Initially, five multilevel models, one for each inflammatory biomarker, were conducted to evaluate the unconditional effect of inflammation at T1 on change in total depressive symptoms. ANOVAs comparing each of these models with and without a random slope for months to follow-up indicated that including a random slope significantly improved model fit; thus, the results reported describe models with a random slope. Log CRP had a small effect on change in total depressive symptoms over time ($\beta = 0.129$, $b = 0.891$, $SE = 0.443$, $p = .046$; see Table S3 in the Supplemental Material) such that higher log CRP at T1 predicted increases in total depressive symptoms over time (following the guidelines for interpreting standardized betas as effect sizes; Acock, 2014). This result was not robust to Bonferroni corrections (adjusted p value = .01). Neither IL-6, IL-8, IL-10, nor TNF-α had significant main effects on change in total depressive symptoms in these models ($p = .077$, $p = .550$, $p = .697$, $p = .112$, respectively).

Next, each model was rerun including the three-way interaction between inflammation, sex, and months to follow-up (see Table S4 in the Supplemental Material). The models with log IL-6, log TNF-α, and log IL-8 had significant T1 Inflammation × Months to Follow-Up × Sex interactions that ranged from medium to large in size ($\beta = 0.473$, $b = 0.333$, $SE = 0.165$, $p = .045$; $\beta = 1.991$, $b = 0.921$, $SE = 0.422$, $p = .031$; $\beta = 1.219$, $b = 0.463$, $SE = 0.222$, $p = .039$, respectively). These results were not robust to Bonferroni corrections (adjusted p value = .01). The three-way interactions were not significant in the models that included CRP or IL-10 ($p = .498$, $p = .577$, respectively). To probe these interactions, significant conditional models were rerun with months to follow-up centered at the minimum value and each quartile (minimum = 0.07 months, first quartile = 8.28 months, second quartile = 13.32 months, third quartile = 19.47 months, fourth quartile = 30.49 months) and with the sex variable centered at male and then recentered at female.

Results indicated that low levels of log IL-6 predicted decreases in total depressive symptoms and high levels of log IL-6 predicted increases in total depressive symptoms at the second, third, and fourth quartiles of months to follow-up for females but not males (see Fig. 1 and Table S5 in the Supplemental Material). Log IL-6 had moderate main effects on change in total depressive symptoms at the second quartile ($\beta = 0.219$, $b = 3.507$, $SE = 1.404$, $p = .013$) and third quartile ($\beta = 0.348$, $b = 5.563$, $SE = 1.829$, $p = .002$) and a large effect at the fourth quartile ($\beta = 0.578$, $b = 9.247$, $SE = 2.824$, $p = .001$) of months to follow-up for females. The TNF-α × Months to Follow-Up × Sex interaction effect also was probed at each quartile of months to follow-up (see Fig. 2 and Table S5) for each sex. Log TNF-α had moderate main effects on change in total depressive symptoms at the minimum value of months to follow-up for males ($\beta = 0.294$, $b = 10.365$, $SE = 4.981$, $p = .040$) such that higher log TNF-α predicted increases in total depressive symptoms at the minimum follow-up value for males. For females, log TNF-α had a small effect at the second quartile ($\beta = 0.172$, $b = 6.068$, $SE = 2.724$, $p = .027$) and a moderate effect at both the third quartile ($\beta = 0.263$, $b = 9.245$, $SE = 3.487$, $p = .009$) and fourth quartile ($\beta = 0.424$, $b = 14.938$, $SE = 5.205$, $p = .005$) of

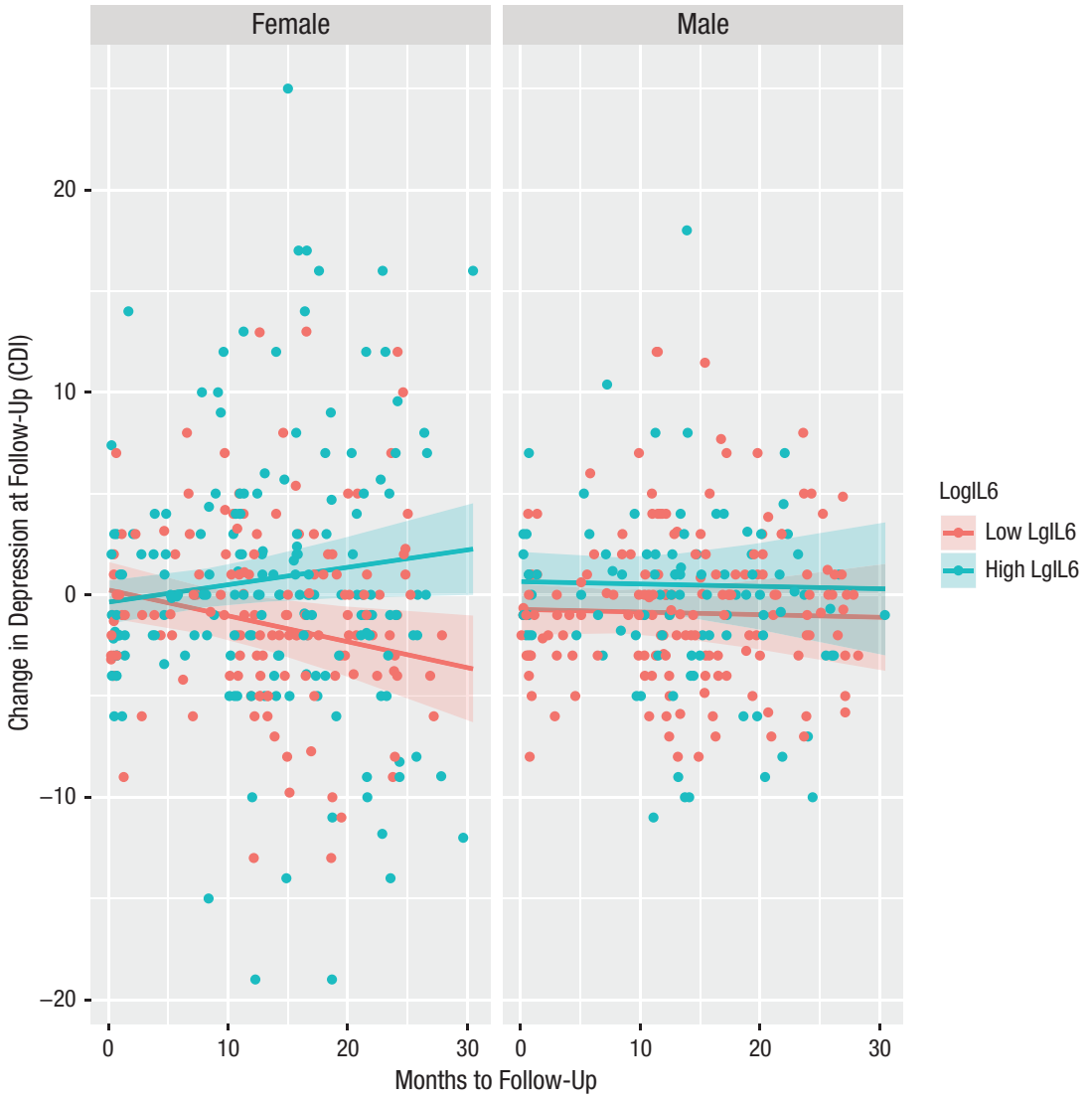

Fig. 1. Log interleukin-6 (IL-6) predicts change in depressive symptoms differentially by sex and months-to-follow-up.

months to follow-up. Higher log TNF-α predicted increased total depressive symptoms at the second through fourth quartiles of months to follow-up for females. When the log IL-8 × Months to Follow-Up × Sex interaction effect was probed, log IL-8 had a moderate effect on changes in total depressive symptoms for males at the fourth quartile, but the relationship was inverse, with higher IL-8 predictive of fewer symptoms (β = −0.385, *b* = −8.513, *SE* = 4.232, *p* = .046; see Fig. 3 and Table S5).

Exploratory analyses

Given findings that inflammation is differentially associated with different types of depressive symptoms (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008), the models used in the primary analyses were rerun predicting to change in depressive symptom subscales. Unconditional models found a weak effect of log IL-8 on somatic symptoms of depression such that more log IL-8 predicted fewer somatic symptoms of depression (β = −0.125, *b* = −1.263, *SE* = 0.631, *p* = .047). Log IL-6 had a small effect on externalizing symptoms (β = 0.152, *b* = 0.759, *SE* = 0.325, *p* = .020) such that more log IL-6 predicted increases in externalizing symptoms over time. Additionally, log CRP had a small effect on lack of personal and social interest symptoms (β = 0.188, *b* = 0.400, *SE* = 0.152, *p* = .009) such that higher log CRP predicted increases in lack of personal and social interest over time. Of these results, only the effect of log CRP on lack of personal and social interest symptoms was robust to Bonferroni corrections (adjusted

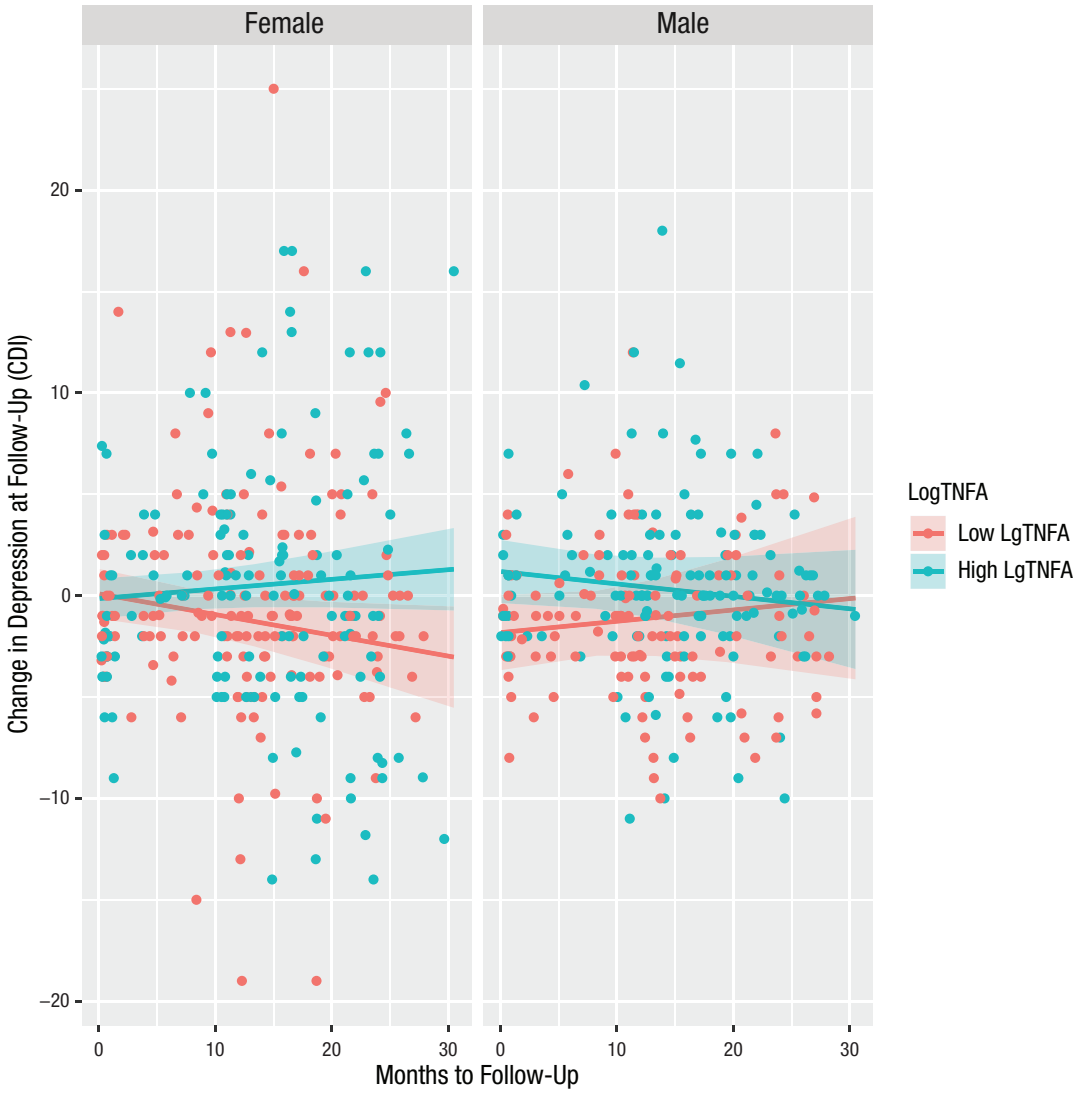

Fig. 2. Log tumor necrosis factor-α (TNF-α) predicts change in depressive symptoms differentially by sex and months to follow-up.

p = .01). There were no other biomarkers that significantly predicted change in subtypes of depressive symptoms, and no biomarkers significantly predicted changes in dysphoria or negative self-concept symptoms (all ps > .05).

Next, conditional models predicting to depressive symptom subtypes were run including the three-way interaction between inflammation, sex, and months to follow-up. Log IL-8 interacted with sex and months to follow-up to have a large effect on changes in somatic symptoms over time (β = 1.432, b = 0.247, SE = 0.098, p = .013). This result was not robust to family-wise Bonferroni corrections (adjusted p = .01). When this interaction was probed using the same procedures described previously, more IL-8 predicted less somatic symptoms for females at the minimum value of months to follow-up (moderate effect; β = −0.323, b = −3.277, SE = 1.063, p = .003; see Fig. S1 in the Supplemental Material) and the first quartile (small effect, β = −0.221, b = −2.238, SE = 0.863, p = .010).

Predicting to externalizing symptoms, the models with log TNF-α and log IL-8 had significant T1 Inflammation × Months to Follow-Up × Sex interactions with large effects on changes in symptoms over time (β = 2.401, b = 0.347, SE = 0.131, p = .009; β = 1.612, b = .0194, SE = 0.069, p = .006, respectively). Both results were robust to family-wise Bonferroni corrections (adjusted p = .01). Specifically, TNF-α predicted more externalizing symptoms for males at the minimum value

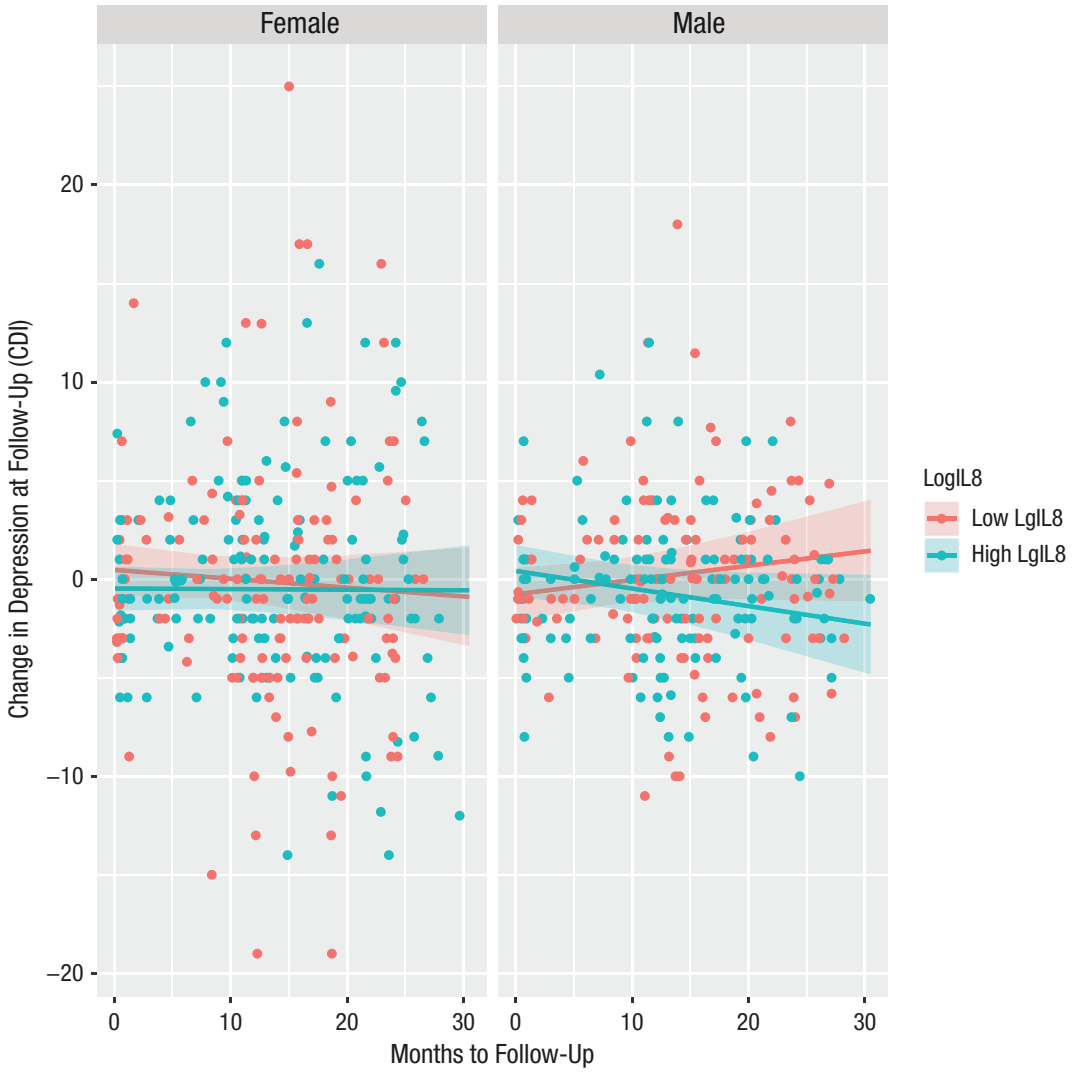

Fig. 3. Log interleukin 8 (IL-8) predicts change in depressive symptoms differentially by sex and months to follow-up.

of months to follow-up (moderate effect, β = 0.452 *b* = 4.966, *SE* = 1.811, *p* = .007; see Fig. S2 in the Supplemental Material). IL-8 predicted more externalizing symptoms at the minimum value of months-to-follow up (small effect, β = 0.234, *b* = 1.637, *SE* = 0.785, *p* = .039; see Fig. S3 in the Supplemental Material) but less externalizing symptoms at the fourth quartile of months to follow-up for males (moderate effect, β = −0.495, *b* = −3.467, *SE* = 1.243, *p* = .006).

With respect to negative self-concept symptoms, the models with log IL-6 and log TNF-α had significant T1 Inflammation × Months to Follow-Up × Sex interactions that ranged from medium to large in size (β = 0.401, *b* = 0.097, *SE* = 0.048, *p* = .046; β = 1.631, *b* = 0.260, *SE* = 0.122, *p* = .036). These results were not robust to family-wise Bonferroni corrections (adjusted *p* = .01). Higher log IL-6 predicted increased negative self-concept symptoms for females at the third (moderate effect; β = 0.282, *b* = 1.551, *SE* = 0.568, *p* = .007; see Fig. S4 in the Supplemental Material) and fourth quartiles of months to follow-up (large effect; β = 0.521, *b* = 2.866, *SE* = 0.817, *p* = .001). Similarly, higher log TNF-α predicted increased negative self-concept symptoms for females at the third (small effect; β = 0.191, *b* = 2.306, *SE* = 1.078, *p* = .034; see Fig. S5 in the Supplemental Material) and fourth quartiles of months to follow-up (moderate effect; β = 0.339, *b* = 4.099, *SE* = 1.474, *p* = .007).

There were no significant three-way interactions predicting to lack of personal and social interest or dysphoria symptoms of depression (all *p*s > .05).

Discussion

Inflammatory proteins predict depressive symptoms conditional on sex and time to follow-up

Our study identified a small main effect of CRP on temporal changes in total depressive symptoms in adolescents as well as interactions between inflammatory cytokines (i.e., IL-6, TNF-α, and IL-8), sex, and time to follow-up on total depressive symptoms. These results are the first to suggest that higher CRP levels are predictive of increases in total depressive symptoms in a general adolescent community sample. Furthermore, the interactions between sex and months to follow-up for IL-6, TNF-α, and IL-8 provide important preliminary evidence of potential methodological considerations for future studies with respect to the required length of follow-up periods. In addition, in keeping with our a priori hypotheses, these relationships with cytokine biomarkers were more evident in females than males. This highlights the need for further research into the biological mechanisms underlying depression in adolescent males.

Post hoc, exploratory analyses found that different biomarkers were differentially predictive of specific subtypes of depressive symptoms, providing further insight into potential sources of heterogeneity among existing studies that use clinical diagnoses or total depressive symptoms without consideration for the types of symptoms endorsed by participants. Our findings also increase the understanding of how inflammation influences specific behaviors that are part of the depressive syndrome. Several of these relationships also were conditional on sex and months to follow-up, with somatic and negative self-concept symptoms of depression predicted by inflammatory biomarkers in females and externalizing depressive symptoms predicted by inflammatory biomarkers in males. These results are particularly interesting given evidence that females tend to show more somatization (Kornstein et al., 2000; Parker, Fletcher, Paterson, Anderson, & Hong, 2014) and lower self-esteem (Bleidorn et al., 2016) than males do. Further, there is evidence that externalizing behaviors might be more characteristic of depression in males compared with females (Gjerde, Block, & Block, 1988; Martin, Neighbors, & Griffith, 2013). In sum, our results provide insight into the nature of the relationship between inflammation and the development of depressive symptoms in adolescence and preliminary evidence of how it may differ between males and females and as a function of specific inflammatory biomarkers and lengths of follow-up.

Specifically, higher levels of IL-6 and TNF-α were associated with increases in total depressive symptoms over time, although the relationship between IL-6 and changes in total depressive symptoms was significant only for females. It also was striking that the association became more evident following the passage of time rather than immediately contemporaneous with the blood sample collection. This finding may reflect the increased prevalence of depression post-menarche in females as well as a summative effect of the sustained bidirectional effects of inflammation and depression (Duivis et al., 2015; G. E. Miller & Cole, 2012). Further, it could be evidence that inflammatory biomarkers influence behavior through the concept of allostatic loading and inflammation's effect on depressive symptoms increases over time. Statistically speaking, there also was less variance in the change scores for depressive symptoms at shorter follow-up intervals. This analysis also uncovered some unique findings for individual cytokines, suggesting that one should be selective in choosing biomarkers for inflammatory panels. Specifically, higher levels of IL-8 were unexpectedly associated with a decrease in total depressive symptoms in males. Functionally, IL-8 has very different immune actions than the other inflammatory cytokines and is more typically associated with neutrophil functions rather than monocytes and lymphocytes. Thus, although IL-8 also is associated with proinflammatory states, its relation to behavior might differ from other proinflammatory cytokines. In addition, there has been one report that IL-8 can be associated with symptoms of mania, and many manic symptoms (e.g., energy, concentration) manifest inversely as depressive symptoms (O'Brien, Scully, Scott, & Dinan, 2006). Further, this result could be an example of Type I error.

In addition to demonstrating the predictive association of inflammatory biomarkers with changes in depressive symptoms over time, the IL-6 and TNF-α results support the theory that individuals with elevated proinflammatory phenotypes may progress to a more chronic course of depressive symptoms (A. H. Miller et al., 2009; G. E. Miller et al., 2002). Furthermore, our results suggest that inflammation is differentially associated with depressive symptoms in females and males. These preliminary findings may help explain the temporal onset and higher prevalence of depression in girls following menarche (Costello et al., 2006; Hankin et al., 1998; Nolen-Hoeksema, 1987; Wade, Cairney, & Pevalin, 2002; Weissman & Keirman, 1977). Finally, the possibility that inflammation is a contributor to depression concurs with the recent interest in employing anti-inflammatory drugs as a therapeutic modality for depression that is resistant to traditional treatments. It

is also consistent with the value of cognitive-behavior therapies, such as mindfulness-based stress reduction (MBSR), which have been found to reduce inflammatory activity and negative emotions in active practitioners (Malarkey, Jarjoura, & Klatt, 2013; Raison et al., 2013; Rosenkranz et al., 2013).

Overall, the findings support that inflammation, specifically CRP, IL-6, TNF-α, and IL-8, were associated with changes in depressive symptoms, but this effect was conditional on both sex and the time to follow-up. This is the first time that these relationships have been found in a community adolescent sample, a group at increased risk for first episode of depression (Hankin et al., 1998). It also should be emphasized that the selection of the specific biological measures is key for this type of study, and the sensitivities and specificities as a bioindicator will be different. In our analyses, the regulatory and anti-inflammatory cytokine, IL-10, did not significantly predict changes in depressive symptoms regardless of whether the model accounted for the potential conditional effects of time to follow-up or sex.

Strengths and limitations

This study had several important strengths. First, it included a large, ethnically diverse sample of adolescents, a group that is still underrepresented in the extant behavioral medicine and health psychology literatures. Second, this study's follow-up design (two 6-month follow-ups per year with every third follow-up scheduled a month after previous study visit) increased the variation in time to follow-up, allowing for a thorough investigation of the moderating effect of months to follow-up. Third, utilizing a sample of adolescents may result in less potential confounds for the immune measures (e.g., medication status, cumulative life stress, chronic illness) compared with an identical study with an adult sample. However, several limitations also should be acknowledged. First, the age for many participants at the time of blood draw was nearing the upper end of the age range for which the CDI was validated. Second, the assessments were based on self-reported depressive symptoms rather than structured interviews and thus did not include diagnosed depression. Although inflammation is more typically associated with depressive symptoms rather than depression diagnoses (which include criteria such as impairment that are not theoretically associated with inflammation; Krishnadas & Harrison, 2016) and thus, symptom-level analyses of these associations are important, the clinical relevance of this study could have been improved through the inclusion of diagnostic interviews for delineating clinically significant depressive episodes. Third, these results do not allow for causal interpretations of the relationship between inflammation and depressive symptoms. Repeated blood draws for the biomarkers used in this study would provide a stronger design allowing for causal interpretations of results as well as tests of the bidirectional relationship between inflammation and depressive symptoms and is a valuable future direction for this research. Additionally, the literature would benefit from a similar study as this one but testing depressive symptoms as the independent variable and inflammation as the dependent variable to explore potential conditional effects of time to follow-up and sex. Fourth, this study would have been improved by collecting additional data that might have influenced the levels of inflammation on the day of the blood draw (e.g., exercise, life stress, sleep). Finally, many results were not robust to Bonferroni corrections and thus should be considered preliminary; however, some of the effects identified were moderate to large in size, supporting their potential relevance to this body of research.

Conclusion

This study provides support for an association between inflammation, specifically CRP, IL-6, IL-8, and TNF-α, and the development of depressive symptoms in a diverse community sample of adolescents. These biomarkers have predictive value for change in depressive symptoms, the strength of which appears to be contingent on the time interval between the assessments of inflammation and depressive symptoms. Most importantly, the relationship between inflammation and depressive symptoms was conditional on sex and more evident in female adolescents. The findings provide insight into some of the antecedents of depression in adolescents and identify a number of useful methodological issues to consider in future research on this question, especially with adolescent participants who are maturing across the course of the evaluation. Furthermore, the linkage between cytokine levels and depression risk is in keeping with the current interest in screening anti-inflammatory medications as adjunct therapeutic modalities for treatment-resistant depression (Raison et al., 2013).

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Author Contributions

D. P. Moriarity generated hypotheses, ran and interpreted analyses, and drafted the manuscript. N. M. Giollabhui

participated in data analysis and provided feedback on the manuscript. L. M. Ellman participated in the design and cleaning of the inflammation data and provided feedback on the manuscript. J. Klugman provided statistical consultation for the project. C. L. Coe assayed blood samples, aided in database construction, and provided feedback on the manuscript. L. Y. Abramson helped design the original study and participated and helped in drafting the manuscript. L. B. Alloy helped design the original study, participated in the design and coordination of this study, and provided feedback on all drafts of the manuscript. All the authors approved the final manuscript for submission.

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Supplemental Material

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