Archival Report

Enhanced Immune Activation Following Acute Social Stress Among Adolescents With Early-Life Adversity

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ABSTRACT

BACKGROUND: Early-life adversity (ELA) has been linked to higher depression risk across the life span and chronic inflammatory conditions that contribute to earlier mortality. In this study, we characterized innate immune responses to acute social stress in a community sample of adolescents (mean age = 13.9 ± 1.6 years; 46.4% female) as a potential pathway linking ELA and depression pathogenesis.

METHODS: Parents reported their child's exposure to 9 ELAs, and adolescents participated in the Trier Social Stress Test for Children, with blood collected immediately before and then at 60 and 90 minutes thereafter. Overall, 65 adolescents had complete data for analysis of stress-induced changes in gene expression and 84 adolescents had complete data for circulating inflammatory markers.

RESULTS: Relative to adolescents exposed to no ELA (11.9%) or low ELA (ELA = 1−3; 67.9%), those exposed to high ELA (ELA = 4+; 20.2%) showed larger stress-associated increases in expression of both proinflammatory and innate antiviral gene transcripts in circulating blood. Consistent with a potential mediating role of sympathetic nervous system activity, promoter-based bioinformatics analyses implicated CREB transcription factor activity in structuring observed gene expression differences. These effects were accompanied by a smaller initial but protracted increase in circulating interleukin 6 in adolescents with high ELA.

CONCLUSIONS: Results are consistent with the hypothesis that ELA may enhance cellular and gene regulatory reactivity to stress, which may, in turn, increase vulnerability to depression and other inflammation-related disease processes.

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Exposure to cumulative early-life adversity (ELA) has been linked to increased risk for depressive disorders (1–3). One common approach to assessing ELA is the adverse childhood experiences (ACE) questionnaire developed by the Centers for Disease Control and Prevention and Kaiser Permanente San Diego (2). In a seminal study using this questionnaire, ACEs were associated with a graded increase in adulthood health risk, with the group exposed to 4 or more ACEs (12.5% of respondents) showing substantially poorer health (2), nearly 4-fold greater risk for depressed affect (4), and at least ninefold greater risk for suicide (2). Health risk among this high exposure group has since been replicated in multiple populations (5). Identifying modifiable risk pathways from ELA to depression in this highest exposure group may lead to substantial mitigation of disease burden for individuals and society.

One putative pathway linking ELA to depression is exaggerated reactivity of the innate immune system leading to chronic inflammation (6). Greater exposure to ELA is associated with elevated inflammation as measured by interleukin 6 (IL-6) and C-reactive protein (CRP) in both pediatric and adult samples (7,8), as well as tumor necrosis factor α (TNF-α)

among adults (8). This may occur in part because species from fish to primates show increased proinflammatory gene expression in monocytes after prolonged exposure to adverse environmental conditions (9). ELA has been linked to tonic upregulation of inflammatory genes among school-aged children (10), adults reared in poverty (11,12), and women with breast cancer (13). However, the immunoregulatory mechanisms explaining exaggerated risk for depression among ELA-exposed individuals are not yet sufficiently understood to guide prevention and remediation.

The innate immune system protects individuals from disease through physical barriers, phagocytic cells, and soluble inflammatory proteins. The accumulation of these inflammatory proteins has been the focus of most research linking ELA to depression (14,15). However, inflammatory protein production is primarily driven by gene activation in specific immune cell populations (particularly monocytes and other myeloid lineage cells). Thus, the biological risk pathways from ELA to chronic systemic inflammation are likely to be more specific to changes in how social stress activates these intracellular processes and in which immune cell subsets they are found

(16). In addition to microbes and tissue damage, the immune system is also activated by uncontrollable social stress (16–18). This effect is mediated by increased activity in the sympathetic nervous system (SNS), monocyte release from the bone marrow and marginal pool into circulation, and adrenergic receptor-mediated changes in gene regulation (19–21). Adults exposed to childhood maltreatment show exaggerated increases in IL-6 (22,23) and exaggerated inflammation-promoting transcriptional control and signaling pathway activation (24) following acute social stress. This immunologic stress reactivity may explain why social stress is a leading risk factor for depressive episodes (25) and other costly, common, and chronic inflammatory syndromes (26).

Innate immune responses to social stress may be especially informative during adolescence, which is a developmental period marked with more frequent social rejection, increased sensitivity to social threat, and increasing risk for depression onset (27,28). Adolescence also involves profound brain circuit (29) and efferent psychophysiological pathway (30) reorganization, which forecasts their functioning into adulthood. Whether ELA-exposed adolescents demonstrate upregulated inflammatory responses to social stress is important to our understanding of when this pattern emerges and the boundary conditions for its malleability. Yet, no study to date has characterized the innate immune response to social stress in adolescents.

This study aimed to characterize one major biological pathway from ELA to depression—increased immunologic reactivity to social stress—for the first time in an adolescent sample. We hypothesized that adolescents exposed to 4+ ELAs would demonstrate an elevated inflammatory response to the Trier Social Stress Test for Children (TSST-C) as measured via intracellular gene regulation, innate immune cell mobilization, and circulating proinflammatory cytokines.

METHODS AND MATERIALS

Participants

Participants were 84 adolescents (44.0% female); 2.4% identified as American Indian/Alaskan Native, 19.0% as Asian, 10.7% as Black/African American, 27.4% as Hispanic/Latinx, 1.2% as Native Hawaiian/Pacific Islander, and 59.5% as non-Hispanic White, with some identifying with multiple categories. Participants were recruited via mass mailing based on census records of households with children between 12 and 15 years. Interested parents completed a phone interview to determine eligibility. Youth were not eligible to participate if they could not read or understand English; had a bleeding disorder (e.g., hemophilia); had any current or past major depressive episode, psychotic symptoms, mania, autism spectrum disorder, or any current chronic medical conditions (e.g., diabetes, cancer); or were regularly taking medications known to influence the immune system (e.g., inhaler, antihistamines, antidepressants). Participants were not excluded based on smoking or alcohol use, although these behaviors were assessed using self-report on the MacArthur Youth Risk Behavior Survey (31,32). Smoking was infrequent in our sample; 14.3% (n = 12) of participants reported ever trying smoking, vaping, or using tobacco products, and only 3.6% (n = 3) participants reported using these products once in the past month. Alcohol use was more frequent; 22.6% (n = 19) of participants reported having

consumed alcohol at some point, but only 6 (7.1%) reported use in the past month.

A total of 97 participants were recruited into the study overall; however, only 86.6% (N = 84) provided all three plasma samples, and 67.0% (n = 65) had usable RNA for both pre- and poststress gene expression assays. The youth with and without blood samples showed no differences in age, body mass index (BMI), ELA, gender, familial home ownership, household income, or ethnicity (all p values > .23).

Procedures

All study procedures were approved by the Los Angeles and Irvine campus Institutional Review Boards of the University of California, and study enrollment took place between August 2017 and March 2020. All participants and their parents provided written informed consent. Data collection for this study occurred in a single laboratory visit beginning between 1:00 and 4:00 PM to control for diurnal variation in immune markers and took place within a clinical research unit. Height, weight, and body temperature were measured by a nurse prior to the catheter insertion, and participants were rescheduled if they showed any signs of infection. Participants abstained from taking any medications for 24 hours before their appointment.

Social stress was induced using the TSST-C (33). This task is a reliable, socially evaluative stress paradigm adapted for use in children from the Trier Social Stress Test (34–36) and took approximately 18 minutes. Blood was collected immediately before and then at 60 and 90 minutes after TSST-C initiation. Participants also provided serial affect ratings every 15 minutes throughout the visit.

Measures

Early-Life Adversity. Adolescents were grouped into epidemiologically meaningful groups (no [ELA = 0], low [ELA = 1−3], and high ELA [ELA = 4+]) based on parent responses to a standardized checklist administered via standardized interview. This 9-item checklist was adapted from the ACE questionnaire (1,2) and included financial insecurity affecting access to food or shelter, emotional abuse, witnessing domestic violence, parent separation/divorce, caregiver mental illness or substance use problems, family member in prison, death of a loved one, loss of home due to natural disaster, and serious personal injury. Use of parent-reported ACEs are consistent with the approaches used by the United States Department of Health and Human Services in the National Survey of Child and Adolescent Well-being [e.g., (37)] and the National Survey of Children's Health [e.g., (38)]. See the Supplement for additional ELA measures and corroboration via adolescent self-report.

Affect. Participants provided serial affect ratings via the Positive and Negative Affect Schedule (39) on arrival, immediately before and after the venous catheter insertion, and every 15 minutes after the TSST began. For each item, participants indicated the extent to which they were feeling any of the following "right now" according to a 5-point Likert scale from "very slightly or not at all" to "extremely." The scale included 10 negative and 10 positive affect items. Negative affect showed acceptable internal reliability, $\alpha > 0.75$, and

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positive affect showed excellent internal reliability, $\alpha > 0.90$, throughout the laboratory protocol.

Gene Expression. Stress-induced changes in gene expression occur within 45 minutes of stress initiation (24); therefore, inflammatory gene expression was assessed in blood collected before stress initiation and 60 minutes after. RNA was quantified using a standard messenger RNA (mRNA)–targeted sequencing assay in the UCLA Neuroscience Genomics Core Laboratory (40). See the Supplement for blood processing and assay procedures.

Circulating Inflammatory Markers. Circulating inflammatory responses to stress are reliably detected 35 to 120 minutes after stress initiation (18). CRP and a five-plex of cytokines that included TNF-α and IL-6 were measured in blood collected immediately before TSST-C initiation and 60 and 90 minutes thereafter. All plasma samples from a participant were assayed on the same plate in duplicate at the UCLA Cousins Center for Psychoneuroimmunology. Interassay coefficients of variation were <10% for CRP and IL-6 and <15% for TNF-α. Mean intra-assay coefficients of variation were ≤5% for CRP and IL-6 and <12% for TNF-α. See the Supplement for blood processing and assay procedures.

Data Analysis

We used univariate analysis of variance or χ^2 to determine whether no, low, or high-ELA groups differed on any demographic or adolescent self-reported ELA and reported F values or Somer's d effect sizes as appropriate for continuous or categorical variables, respectively, in Table 1.

For plasma samples with concentrations below the lower limit of detection (see the Supplement), values equal to one half of the lower limit of detection were assigned. All circulating markers were transformed using the natural log to adjust for skew. Repeated-measures analysis of variance estimated within- and between-person time effects, ELA, and the interaction between time and ELA on circulating CRP, IL-6, and TNF-α. All models were conducted unadjusted then adjusted for BMI, age, gender, race (Asian, Black, Other, White), and ethnicity (Hispanic/Latinx, non-Hispanic), and all models predicting change in circulating inflammatory markers controlled for baseline concentrations.

Transcript abundance values were normalized to 11 standard reference genes (41) and $\log_2$-transformed for linear model analyses relating pre- to poststress changes in gene expression values (repeated measure) to ELA and analyzed using an established approach (40). Primary mixed-effect linear model analyses examined 14 proinflammatory gene transcripts

Table 1. Participant Demographic and Adversity Characteristics by ELA Risk Groups, N = 84

Demographics and Characteristics	ELA Group			Statistic	p
	0, n = 10	1–3, n = 57	4+, n = 17		
Gender, Female	50.0%	47.4%	29.4%	d = 0.13	.21
Age, Years	13.30 ± 1.49	13.89 ± 1.47	14.18 ± 1.63	F = 1.07	.35
BMI, kg/m^2	19.51 ± 2.81	21.69 ± 4.32	21.91 ± 4.62	F = 1.23	.30
Ethnicity, Hispanic/Latino	30.0%	21.0%	47.1%	d = 0.15	.21
Race, Non-Hispanic White	50.0%	70.0%	29.4%	d = 0.19	.11
Household Income <$50,000/Year	0.0%	7.0%	35.3%	d = 0.29	.009[a]
Own Their Home	100.0%	68.4%	41.2%	d = 0.34	<.001[a]
Mother's Highest Education, College Degree or Higher	100.0%	78.9%	64.7%	d = 0.08	.40
Father's Highest Education, College Degree or Higher	100.0%	78.9%	47.1%	d = 0.31	.002[a]
Parents Are Married	100.0%	87.7%	58.8%	d = 0.48	<.001[a]
Nonintentional Trauma Total	3.10 ± 1.45	4.64 ± 2.54	6.24 ± 2.97	F = 5.08	.008[a]
Nonintentional Trauma, Chronicity	4.86 ± 2.48	9.58 ± 7.73	16.17 ± 8.94	F = 5.54	.006[a]
Nonintentional Trauma, Age of Onset	5.10 ± 4.18	4.61 ± 3.26	5.59 ± 3.83	F = 0.53	.59
Physical Trauma Total	1.90 ± 1.37	1.78 ± 1.86	2.94 ± 1.87	F = 2.67	.08
Physical Trauma, Chronicity	3.63 ± 4.03	5.58 ± 7.98	9.25 ± 7.84	F = 1.89	.16
Physical Trauma, Age of Onset	9.43 ± 2.64	7.91 ± 3.30	9.08 ± 2.87	F = 1.10	.34
Emotional Abuse Total	2.50 ± 1.08	2.60 ± 2.22	3.35 ± 2.23	F = 0.89	.42
Emotional Abuse, Chronicity	8.30 ± 4.81	9.98 ± 10.66	12.13 ± 9.02	F = 0.49	.62
Emotional Abuse, Age of Onset	9.22 ± 2.59	8.17 ± 3.43	8.07 ± 4.01	F = 0.38	.69
Sexual Trauma Total	0.00 ± 0.00	0.02 ± 0.13	0.06 ± 0.24	F = 0.58	.56
Sexual Trauma, Chronicity	0.00 ± 0.00	0.09 ± 0.67	0.06 ± 0.24	F = 0.12	.89
Sexual Trauma, Age of Onset	–	11.0 ± NA	14.0 ± NA	–	–

Values are presented as % or mean ± SD.

Nonintentional, physical, emotional, and sexual trauma were assessed via adolescent self-report on the ETI. Totals reflect the number of endorsed events in the subscale and chronicity reflects number of events multiplied by their reported occurrences (1, 2, 3, 4, 5 or more times).

BMI, body mass index; ELA, early-life adversity; ETI, Early Trauma Inventory; NA, not applicable.

[a]Statistically significant group differences ($p < .05$).

(identified from a 19-gene prespecified indicator set after removal of 5 genes showing minimal expression variance in this sample; SD < 0.5 $\log_2$ units) and 25 gene transcripts involved in innate antiviral responses (from 34 prespecified antiviral genes after removal of 9 showing minimal expression variance in this sample) (40). Secondary analyses used TELiS promoter-based bioinformatics analyses to identify genes showing greater than 4-fold change over time in participants with high ELA relative to other groups, testing for differential transcription factor–binding motif prevalence for two transcription control pathways hypothesized to mediate acute social stress effects on gene expression—the CREB factors family involved in beta-adrenergic signaling from the SNS (assessed by TRANSFAC V\$CREB_01 position-specific weight matrix), and the glucocorticoid receptor (V\$GR_Q6) involved in cortisol signaling from the hypothalamic-pituitary-adrenal axis (9,12,24,42,43). All models were tested unadjusted then adjusted for age, gender, race/ethnicity, and BMI.

RESULTS

Adolescents were reported by a parent to have been exposed to between 0 and 7 events of ELA ($mean_{ELA}$ = 2.11, SD_{ELA} = 1.57); 11.9% had no ELA, 67.9% had low ELA, and 20.2% had high ELA. High-ELA youth lived in families with lower household incomes, were less likely to own their home, had fathers without a college degree, and self-reported more exposure to nonintentional traumatic events with greater chronicity. Table 1 details demographic, socioeconomic, and trauma exposure information by ELA group.

There were no statistically significant changes in positive or negative affect from immediately before to immediately after the venous catheter insertion (t_{96} = −0.88, p = .19; t = 0.37, p = .36). Positive affect decreased (t_{96} = 4.40, p < .001) and negative affect increased (t_{96} = −4.71, p < .001) from immediately before the start of the TSST to immediately after its completion. This pattern of affective reactivity to the TSST did not significantly differ between ELA groups (negative affect: $F_{2,78}$ = 2.94, p = .06; positive affect: $F_{2,78}$ = 2.63, p = .08).

Social Stress–Induced Changes in Circulating Inflammatory Markers

Table 2 provides descriptive information for each circulating immune marker across the protocol by ELA group. After accounting for age, gender, BMI, and race/ethnicity, IL-6 increased ($F_{1.77,116.55}$ = 4.34, p = .019) from pre- to poststress. By contrast, CRP ($F_{1.62,107.06}$ = 0.92, p = .38) and TNF-α ($F_{2,132}$ = 1.40, p = .25) did not change across the protocol.

Circulating IL-6 response to stress also differed by ELA (IL-6, $F_{3.41,107.29}$ = 3.56, p = .017), such that youth exposed to no ELA showed a robust increase in IL-6 at 60 minutes after stress that plateaued at 90 minutes. In contrast, youth exposed to high ELA showed a less robust increase in IL-6 that continued to rise from 60 to 90 minutes after stress. Figure 1A displays IL-6 across the stress protocol by ELA and Figure 1B displays change in IL-6 from baseline to 60 and 90 minutes after stress by ELA. ELA was not associated with within-subject changes in CRP ($F_{3.27,102.96}$ = 0.21, p = .91) or TNF-α ($F_{4,126}$ = 1.84, p = .13).

We then conducted a post hoc analysis to determine whether adolescent self-reports of ELA onset or chronicity explained any additional variance in IL-6 response to the TSST. We saw no evidence that accounting for self-reported chronicity or age of onset of these ELA items explained additional variance above and beyond the a priori groupings made based on parent reports at either 60 or 90 minutes (all ΔR^2 < 0.025, p values > .18).

### Social Stress–Induced Changes in Innate Immune Gene Expression by ELA

In the 65 participants for whom pre–TSST-C and 60 minutes post–TSST-C mRNA samples were obtained, primary gene expression analyses examined changes from pre- to 60 minutes post-stress in 14 proinflammatory gene transcripts and 25 gene transcripts involved in innate antiviral responses, examining differences between high, low, and no ELA, unadjusted and then adjusted for race, ethnicity, BMI, age, and gender. In an unadjusted model, expression of the inflammatory gene subset increased following stress (b = 0.14, SE =

Table 2. Raw Circulating Inflammatory Markers Throughout Laboratory Stress Protocol

Inflammatory Markers	Range	ELA = 0, n = 10, Mean ± SD	ELA = 1–3, n = 57, Mean ± SD	ELA 4+, n = 17, Mean ± SD	Overall, n = 84, Mean ± SD
IL-6, pg/mL	0.1–6.7				
Prestress	–	0.5 ± 0.3	0.8 ± 0.9	0.5 ± 0.3	0.7 ± 0.8
60 min after stress onset	–	0.9 ± 0.5	0.7 ± 0.5	0.6 ± 0.5	0.7 ± 0.5
90 min after stress onset	–	0.8 ± 0.5	0.8 ± 0.5	0.7 ± 0.5	0.8 ± 0.5
TNF-α, pg/mL	0.7–2.3				
Prestress	–	1.3 ± 0.2	1.4 ± 0.3	1.1 ± 0.2	1.3 ± 0.3
60 min after stress onset	–	1.3 ± 0.2	1.3 ± 0.3	1.1 ± 0.2	1.3 ± 0.3
90 min after stress onset	–	1.3 ± 0.2	1.3 ± 0.3	1.1 ± 0.2	1.3 ± 0.3
C-Reactive Protein, mg/L	0.10–19.20				
Prestress	–	0.75 ± 0.98	0.84 ± 1.42	2.24 ± 4.79	1.12 ± 2.49
60 min after stress onset	–	0.75 ± 0.92	0.82 ± 1.40	2.07 ± 4.29	1.07 ± 2.31
90 min after stress onset	–	0.72 ± 0.88	0.75 ± 1.36	2.17 ± 4.43	1.04 ± 2.34

Raw concentrations are reported, although inflammatory markers were transformed using the natural log transformation for all analyses.
ELA, early-life adversity; IL-6, interleukin 6; TNF-α, tumor necrosis factor α.

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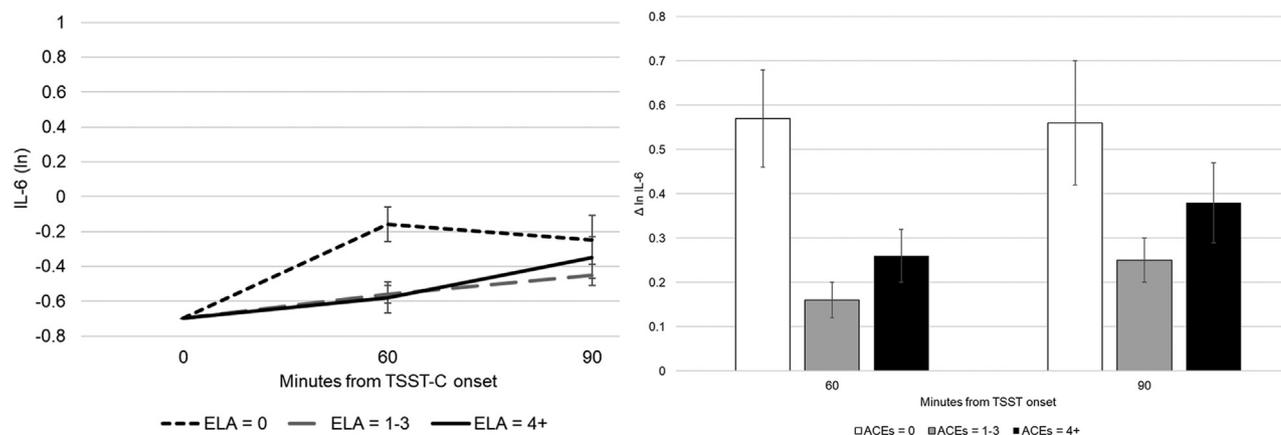
**Figure 1.** Estimated marginal mean (±SE) of circulating interleukin 6 (IL-6) and change in circulating IL-6 following acute social stress, stratified by early-life adversity (ELA) group. Left panel illustrates results of repeated-measures analysis of variance by showing the estimated marginal means of the natural log of IL-6 at 0, 60, and 90 minutes after Trier Social Stress Test for Children (TSST-C) initiation by ELA group. Right panel illustrates change in natural log of IL-6 from 0 to 60 and 0 to 90 minutes after TSST-C initiation by ELA group. Together, participants exposed to no ELA exhibited a significant increase in IL-6 from 0 to 60 minutes, which showed no change between 60 and 90 minutes. In comparison, participants exposed to high ELA showed an attenuated increase in IL-6 from 0 to 60 minutes, but a significant increase in IL-6 from 60 to 90 minutes. IL-6 concentrations for participants with no and high ELA at 90 minutes after TSST-C initiation were not significantly different. All analyses account for gender, age, race, ethnicity, and body mass index. ACE, adverse childhood experience.

0.04, $p = .002$), which remained after accounting for covariates ($b = 0.12$, SE $= 0.04$, $p = .009$). Expression of the antiviral gene subset also increased following stress ($b = 0.07$, SE $= 0.03$, $p = .05$) but was not robust to accounting for covariates ($b = 0.06$, SE $= 0.03$, $p = .07$). Youth with high ELA showed a statistically significant increase in inflammatory gene expression ($b = 0.30$, SE $= 0.10$, $p = .003$), as well as a marginal increase in antiviral gene expression ($b = 0.15$, SE $= 0.08$, $p = .05$). By contrast, youth exposed to low ELA showed no statistically significant change in inflammatory ($b = 0.07$, SE $= 0.05$, $p = .20$) or antiviral gene expression following stress ($b = 0.03$, SE $= 0.04$, $p = .43$). Youth with no ELA also showed no statistically significant change in either inflammatory ($b = 0.09$, SE $= 0.13$, $p = .51$) or

antiviral gene expression following stress ($b = 0.09$, SE $= 0.10$, $p = .41$). Figure 2 displays ELA-related differential change in inflammatory (Figure 2A) and innate antiviral (interferon) gene (Figure 2B) expression following acute psychosocial laboratory stress.

To identify transcription control pathways that might mediate the observed ELA-related differences in inflammatory and antiviral gene expression responses to acute stress using the prespecified composite scores above, we conducted promoter-based bioinformatics analyses of all 381 gene transcripts (genome-wide) showing greater than 4-fold differential change over time among those with high ELA (>3) versus others (0–3: 227 transcripts upregulated and 154
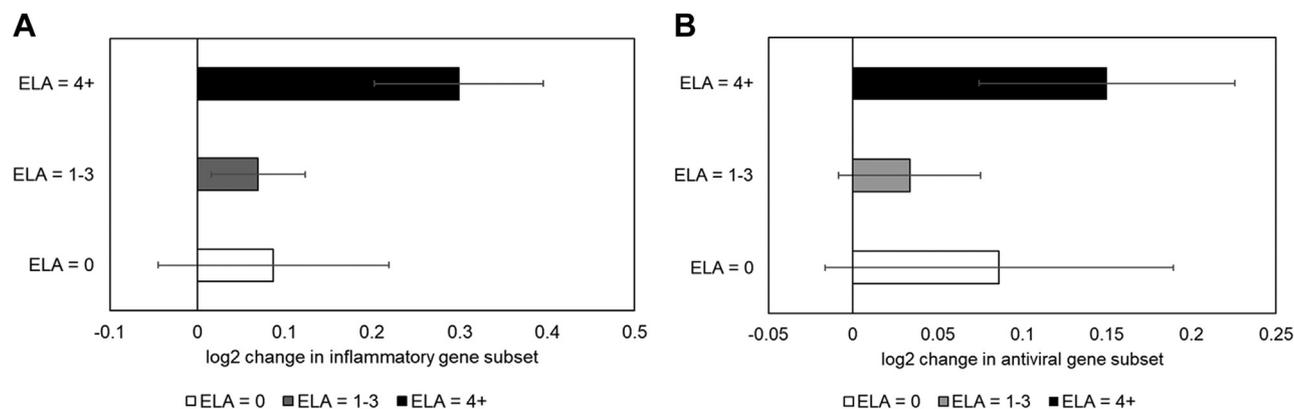
Figure 2. Change in inflammatory and antiviral (interferon) gene subset activation from prestress to 60 minutes after stress by early-life adversity (ELA). For both panels, the x-axis displays change (after Trier Social Stress Test for Children − before Trier Social Stress Test for Children) in gene expression. **(A)** Change in expression of 14 proinflammatory gene transcripts (identified from a 19-gene prespecified indicator set after removal of 5 genes showing minimal expression variance in this sample; SD < 0.5 log$_2$ units). Differential activation of inflammatory gene expression by ELA group was robust to controlling for age, gender, race, ethnicity, and body mass index. Results of the adjusted models are shown. **(B)** Change in expression of 25 gene transcripts involved in innate antiviral responses (from 34 prespecified antiviral genes after removal of 9 genes showing SD < 0.5 log$_2$ units). Differential activation of antiviral gene expression by adverse childhood experiences risk was not robust to controlling for age, gender, race, ethnicity, and body mass index. Results of the adjusted models are shown.

downregulated). Consistent with a potential role for SNS activation in mediating the differential high ELA responsiveness, there was significant over-representation of CREB transcription factor–binding motifs in the promoter DNA sequences of the 227 genes relatively upregulated compared with the 154 downregulated genes (3.14-fold difference; 1.65 $\log_2$ fold difference $\pm$ 0.28 standard error, $p < .001$). This analysis found no differential prevalence of glucocorticoid receptor transcription factor–binding motifs (0.88-fold; -0.18 ± 0.27, $p = .51$).

To determine whether changes in leukocyte subset abundance represented a mechanism of the differential changes in gene expression observed across ELA groups, we conducted additional analyses of inflammatory and antiviral gene expression composites that controlled for leukocyte subsets (using mRNA levels of CD14, CD16, CD3D, CD4, CD8A, CD19, and CD56). In these analyses on a subset of 65 subjects with complete mRNA data, adolescents with high ELA continued to show significant increases in inflammatory ($b = 0.29$, SE = 0.10, $p = .004$) and antiviral gene expression ($b = 0.16$, SE = 0.04, $p = .04$), whereas adolescents with low and no ELA showed smaller, nonsignificant changes (inflammatory: $b = 0.10$, SE = 0.05, $p = .06$ and $b = 0.23$, SE = 0.14, $p = .11$, respectively; antiviral: $b = 0.06$, SE = 0.05, $p = .19$ and $b = 0.17$, SE = 0.11, $p = .15$, respectively). Cellular changes were further explored in an additional subset of subjects ($n = 37$) with flow cytometry data (see the Supplement).

DISCUSSION

To our knowledge, this is the first study to characterize the intracellular and circulating innate immune response to acute social stress in an adolescent sample. Adolescents exposed to 4 or more ELAs demonstrated enhanced immune responses to acute social stress compared with those with no or low ELA, including increased expression of proinflammatory genes, and a smaller initial but more protracted increase in plasma IL-6. Bioinformatic analyses indicated increased activity of CREB transcription factors as a potential mechanism for enhanced transcriptional responsiveness of adolescents with high ELA. CREB transcription factors mediate SNS activation via beta-adrenergic receptors. Parallel bioinformatic analyses showed no indication of increased glucocorticoid receptor activity, suggesting a dominant role for the SNS rather than the hypothalamic-pituitary-adrenal axis in structuring the enhanced transcriptional responses. This may be consistent with observations that adversity-exposed youth experience social-evaluative stressors as more of a threat than a challenge, which differentially engages autonomic nervous activity [44]. Together, these results identify increased reactivity of the innate immune system to acute social stress in adolescents exposed to high ELA, thus providing a potential immunoregulatory pathway through which ELA might interact with stressful events in adolescence to enhance individual vulnerability to depression and other diseases associated with chronic inflammation.

One study to date has demonstrated an increase in proinflammatory gene expression following acute laboratory stress among healthy adults (aged 45–60) with a history of ELA [24]. This study extended these past observations to an adolescent

sample and demonstrated that youth exposed to high ELA were susceptible to greater intracellular immune activation following social stress, despite showing little evidence of exaggerated increases in circulating inflammatory proteins. Convergence of these findings across adolescents and adults is important given that ELA is more distal for adult participants and therefore may indicate that these inflammatory processes persist with time. Exaggerated innate immune activation among ELA-exposed youth has important implications for how stress becomes biologically embedded. Stress generation is the process by which negative experiences perpetuate more stressful experiences and contribute to diseases such as depression [45,46]. Exaggerated stress-induced transcriptional immune activation may lead to stress generation through inflammation-related social withdrawal [47], decreased motivation, and exaggerated negative affective reactivity [48], while increased signal transduction may also increase susceptibility to the behavioral correlates of inflammation among ELA-exposed individuals [49]. Clarifying this mechanism of risk is essential to distinguishing factors that predict individual-level versus group-level risk for health disparities [5].

Contrary to our hypotheses, as well as observations in adults [22], ELA was associated with a smaller initial but a more protracted increase in IL-6 following the TSST-C. The discrepancy in findings could have been the result of time since ELA exposure, suggesting that ELA-related changes to circulating inflammatory reactivity show a delayed pattern across development that could perhaps depend upon or follow intracellular changes. Alternatively, the seminal study in adults (ages 18–64 years) [22] focused on childhood maltreatment while this study included more diverse experiences of ELA; thus, maltreatment may specifically contribute to the magnitude of the initial circulating inflammatory response to stress while other forms of ELA shape immunoregulatory processes differently [50]. Increases in inflammatory markers following stress can result from multiple sources [18]: blood pressure–related decreases in total circulating plasma, increases in inflammatory protein-synthesizing cells, and increases in protein synthesis within cells and tissues. This protracted pattern of IL-6 increase relative to the group with no ELA may suggest that their IL-6 concentrations came from different sources. Notably, the final blood sample was 90 minutes after stress onset, although stress-induced changes to immune gene expression can persist for 180 minutes [24]. A more delayed sample may have shown cytokine concentrations that continued to increase for the high-ELA group but recovered quickly for others. That being said, CRP was elevated in our high-ELA group at baseline, which is consistent with meta-analytic evidence in both pediatric and adult populations [7,8]; thus, any stress-induced increase in inflammation was adding to that difference in baseline systemic inflammation. The combination of a protracted stress response, slight elevations in more stable systemic inflammatory proteins such as CRP, and more frequent exposure to social stress may explain elevations in systemic inflammation that emerge in adolescence [7] and persist into adulthood [8] among ELA-exposed individuals.

CRP, IL-6, and TNF-α have all been associated with both ELA and depression [8,51]. We did not observe any evidence of CRP or TNF-α reactivity. This was consistent with the literature; specifically, IL-6 shows the most robust and

consistent reactivity to acute stress, followed by CRP, while TNF-α is not reliably responsive ([18]). Indeed, the time course of changes in CRP should be more delayed relative to IL-6 because it is synthesized in the liver in response to IL-6 ([52,53]). This may explain why increases in CRP occur on the order of hours and days ([52,54]), rather than minutes, following an immune stimulus. Why TNF-α is not typically responsive to psychosocial stress is less clear, because it is synthesized by similar leukocyte populations as part of the innate immune response, although there is some evidence that TNF-α stimulation can be suppressed by stress ([55,56]).

Differential immune responses to social stress may extend our understanding of how ELA leads to depression. Previous studies have largely proposed a model whereby ELA leads to elevated inflammatory proteins in the periphery and central nervous system following pathogen exposure or stress, which compounds over time into chronic systemic inflammation ([6,7,16]). The empirical support for this model is strongest among ELA-exposed individuals who are already depressed or experiencing elevated symptoms ([57]), not necessarily among ELA-exposed adolescents prior to depression onset. ELA may be specifically promoting CREB signaling within immune cells. CREB is a multifunctional transcription factor that can be both pro- or anti-inflammatory, prevents apoptosis in monocytes and macrophages, and promotes immune cell proliferation and differentiation ([58]). Over time, this may contribute to shifts in the cellular composition of the immune system that serve to perpetuate inflammatory signaling in both the periphery and the central nervous system. Differential sensitivity and activation of CREB may also help to explain why ELA-exposed individuals demonstrate greater sensitivity to depressive symptoms following an immune challenge ([49]), despite showing a smaller initial inflammatory response to acute stress as we observed in this sample. Together, altered sensitivity of CREB transcription factor activity following social stress among ELA-exposed individuals may be the indirect pathway through which ELA ultimately presages elevated systemic inflammation over time.

These results should be considered in the context of the study's limitations. ELA was intentionally oversampled for in this study because only 5% to 12% of the general population is exposed to high ELA ([2,3,59,60]). However, ELA exposure was still not normally distributed in the sample; about 10% and 20% of participants were exposed to no and high ELA, respectively. Replication in a sample balanced across these ELA groups would help to rule out spurious results that occur as an artifact of imbalanced groups. Replication in a sample with a wider range of BMI may also be informative. BMI was not a statistically significant predictor of circulating or intracellular immune reactivity, yet only 14% of our participants were overweight and 4% were obese. BMI's role may be more prominent in samples where more obese individuals are represented. Our mechanistic conclusions are limited by the absence of additional, serial measures of autonomic nervous activity, such as concentrations of norepinephrine or epinephrine in blood, galvanic skin response, or heart rate. Our conclusions may also be limited in that the differentially expressed genes identified in our RNA sequencing analyses were not further corroborated by real-time reverse transcriptase polymerase chain reaction. However, our approach has shown convergent validity with polymerase chain reaction validation in the past ([61]). Finally, there were several sources of missing immune data: participants refusing the catheter after enrollment, low blood flow, hemolysis, and centrifuge malfunction. Whether these or other sources of missing data reflect a meaningful sampling bias is unclear.

The high-ELA group responded to social stress with a larger increase in innate immune gene expression and greater CREB transcriptional activity. Psychosocial stressors may lead to depression through the compounding effects of more frequent stressful experiences and an altered immune transcriptional response to daily stressors, which both is consistent with and extends the biological embedding hypothesis ([62,63]). The effects of acute social stress on immune gene expression can be blocked pharmacologically ([64]) and there is accumulating evidence that peripheral immune dysregulation can be mitigated by psychosocial interventions ([65,66]), although none of this research has yet to be conducted among adolescents. Given that adolescence is a period of heightened social rejection, increased social threat sensitivity, and increasing risk for depression onset ([27,28]), there is an urgent need for mechanistic research in this population.

ARTICLE INFORMATION

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