

ORIGINAL ARTICLE

Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood

E Chen¹, GE Miller¹, MS Kobar^{2,3} and SW Cole^{4,5,6}

¹Department of Psychology, University of British Columbia, Vancouver, BC, Canada; ²Centre for Molecular Medicine & Therapeutics, Child and Family Research Institute, Vancouver, BC, Canada; ³Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; ⁴Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA, USA; ⁵UCLA AIDS Institute, Molecular Biology Institute, Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA and ⁶UCLA Norman Cousins Center, University of California, Los Angeles, CA, USA

The notion that family support may buffer individuals under adversity from poor outcomes has been theorized to have important implications for mental and physical health, but little is known about the biological mechanisms that explain these links. We hypothesized that adults who grew up in low socioeconomic status (SES) households but who experienced high levels of maternal warmth would be protected from the pro-inflammatory states typically associated with low SES. A total of 53 healthy adults (aged 25–40 years) low in SES early in life were assessed on markers of immune activation and systemic inflammation. Genome-wide transcriptional profiling also was conducted. Low early-life SES individuals who had mothers, who expressed high warmth toward them, exhibited less Toll-like receptor-stimulated production of interleukin 6, and reduced bioinformatic indications of pro-inflammatory transcription factor activity (NF- κ B) and immune activating transcription factor activity (AP-1) compared to those who were low in SES early in life but experienced low maternal warmth. To the extent that such effects are causal, they suggest the possibility that the detrimental immunologic effects of low early-life SES environments may be partly diminished through supportive family climates.

Molecular Psychiatry (2011) 16, 729–737; doi:10.1038/mp.2010.53; published online 18 May 2010

Keywords: socioeconomic status; maternal warmth; immune

Introduction

Individuals lower in socioeconomic status (SES) consistently suffer poorer mental and physical health. They are at heightened risk for numerous mental health problems, including depression, anxiety and disruptive disorders,^{1,2} as well as for a variety of chronic medical illnesses, including cardiovascular disease, arthritis and some cancers.^{3,4} These relationships are robust across the life span,^{5,6} as well as across both countries that do and do not have universal health-care systems.⁷ These patterns make low SES one of the most robust risk factors for disease and disability.

Researchers have long speculated, however, that there are individuals who are able to thrive, despite confronting serious adverse circumstances such as being low in SES. This is the notion of resilience; that

is, individual characteristics or situational processes that can buffer those facing adversity from poor outcomes.^{8,9} In children, the types of factors that have most consistently been found to be protective against outcomes such as mood and antisocial disorders include supportive relationships with others such as close family relationships and authoritative parenting.^{10–13}

What has remained less clear, however, are the biological processes by which supportive families may protect against disease and disability related to adversity. One of the leading mechanistic hypotheses about how SES exerts its effects on health is by inflammation.¹⁴ Research shows, for example, that in healthy adults, low SES is associated with systemic inflammatory profiles, such as elevated levels of C-reactive protein (CRP) and interleukin 6 (IL-6) in circulation.^{15,16} In children, low SES is associated with increased cytokine production in response to *in vitro* mitogen challenge in patients with inflammation-related diseases such as asthma.^{17,18} More recently, functional genomics studies have indicated that several different types of social adversity, including low SES, social isolation and chronic

Correspondence: Dr E Chen, Department of Psychology, University of British Columbia, 2136 West Mall, Vancouver, BC V6T1Z4, Canada.

E-mail: echen@psych.ubc.ca

Received 5 October 2009; revised 17 March 2010; accepted 12 April 2010; published online 18 May 2010

stress, are all associated with heightened signaling of pro-inflammatory transcription control pathways in both children and adults.^{19–23}

In turn, inflammation has been implicated as a key pathway in numerous illnesses ranging from depression to cardiovascular disease.^{24–27} For example, inflammation is known to contribute to the progression of coronary heart disease by facilitating the growth and rupturing of atherosclerotic plaque, and activating coagulation processes involved in thrombogenesis.^{26,28} Mounting evidence indicates that inflammatory cytokines can also induce depressive symptoms.^{29,30} Administration of inflammatory cytokines evokes a constellation of sickness behaviors that resembles depression, including anhedonia, social withdrawal, anorexia and hyposomnia.³¹ In adults, those who are administered inflammatory cytokines as part of medical treatments are at greater risk for experiencing depressive symptoms, and this in turn can be prevented prophylactically by administering antidepressants.³²

Although low SES is associated with a pro-inflammatory profile, researchers have argued that it, and other physiological concomitants of adversity, can be offset by individual characteristics or supportive processes.^{33–35} No studies that we are aware of, however, have directly tested this hypothesis. Further, it is not known whether any buffering effects of positive early-life factors could have lasting effects on biology into adulthood (the focus of previous research has been on current psychosocial factors that mitigate the biological profile of adversity in children and in adults^{36–38}). In the animal literature, high levels of maternal licking/grooming and arched back nursing early in life buffer rats from elevated physiological responses to stressors later in life as adults.^{39–41} These findings suggest that early-life maternal care may serve as one type of support factor that protects against detrimental biological responses in adult life.

In this study, we hypothesized that among adults who faced adverse circumstances early in life (low SES), those who experienced high levels of maternal warmth in childhood would show reduced indications of pro-inflammatory signaling compared to those who experienced low maternal warmth. More specifically we hypothesized that as adults, low early-life SES individuals who experienced high maternal warmth would show (1) reduced activity of a key pro-inflammatory transcription factor, nuclear factor- κ B (NF- κ B), in bioinformatic analyses of genome-wide transcriptional profiles of peripheral blood mononuclear cells (PBMCs); (2) reduced PBMC responsiveness to *in vitro* stimulation, as indicated by lower expression of the pro-inflammatory cytokine IL-6 and (3) reduced systemic inflammation, as indicated by lower levels of circulating CRP. We tested secondary hypotheses that other transcription control pathways involved in immune activation, such as AP-1, would also show indications of reduced activity in low early-life SES individuals who experienced high maternal warmth in childhood.

Methods

Sample

Participants were 53 adults recruited from Vancouver, BC through postings in local media and public transit. To be eligible, they had to be 25–40 years of age and in good health, defined as being free of infection the past 4 weeks and without a history of psychiatric or chronic physical illnesses (by self-report). Participants also had to have been raised in a low SES household in early life (details below). Of these 53 participants, 26 were in the high-maternal-warmth group and 27 were in the low-maternal-warmth group (details below). Participants represent the low SES subset of a larger study, described in more detail by Miller *et al.*²² The project was approved by the University of British Columbia's Research Ethics Board and all subjects gave written consent before participating.

Socioeconomic status

SES was defined according to occupational status using the United Kingdom's National Statistics Socio-economic Classification.⁴² This study focused on individuals who fell into the low SES category, based on their parents having routine, manual or lower supervisory occupations. For classification of early-life SES, we contacted each subject's mother or father by telephone, and inquired about their occupation (and their spouse's) during their child's first 5 years of life (including the *in utero* period). Given that social status is often ascribed to households rather than individuals, we used the highest occupation in the family to categorize SES, a common approach in the SES literature.⁴³ Average early-life SES for this sample was 6.0 (s.d. = 1.3) on a 1–7 scale, with lower numbers indicating lower prestige occupations.

Maternal warmth

Maternal warmth experienced in childhood was measured using the Parent Bonding Inventory.⁴⁴ This inventory consists of a subscale assessing maternal warmth/care. This scale consists of 12 items querying the quality of their relationship with their mother during childhood (for example, 'My mother spoke to me in a warm and friendly voice.'). Participants reported on a four-point scale how true each statement was of their own experiences. The Parent Bonding Inventory has shown adequate test-retest reliability (0.60–0.79), and has shown adequate validity when scores were compared between twins, as well as with interview-based ratings.⁴⁵ In addition, it has been used on other studies investigating family relationships and physiological measures.^{46,47} In our studies, internal consistency of this scale was 0.94, and 6-month test-retest reliability was 0.84. Participants in the top half of the score distribution were coded as high maternal warmth ($n = 26$), and those in the bottom half were coded as low maternal warmth ($n = 27$).

Transcriptional profiles

PBMCs were isolated from whole blood through density-gradient centrifugation, lysed and then homogenized in QiaShredder Spin Columns (Qiagen, Mississauga, ON, USA). Lysates were frozen at -80°C until RNA was extracted using AllPrep DNA/RNA kits (Qiagen). RNA purity and integrity were verified using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA (50 ng) was later assayed on an Illumina BeadStation 500 using HumanRef-8 v3.0 Expression BeadChips (Illumina, San Diego, CA, USA) for a randomly selected subgroup of 30 low early-life SES individuals (15 high and 15 low maternal warmth). The assays were performed at the Génome Québec Innovation Centre. The raw data were log-2 transformed and differentially expressed genes were identified as those showing $\geq 20\%$ difference in mean expression levels between low- and high-maternal-warmth groups (corresponding to a false discovery rate of $< 5\%$ (Cole *et al.*⁴⁸)). The data are deposited in Gene Expression Omnibus (accession number: GSE15180).

To identify the upstream signal transduction pathways that underlie differential gene expression, we used a two-sample variant of the Transcription Element Listening System (<http://www.telis.ucla.edu>).⁴⁹ Transcription Element Listening System analyzes differential gene expression data in terms of the prevalence of transcription factor-binding motifs (TFBMs) within the promoters across all differentially expressed genes. This approach can accurately identify the activation of specific hormone or cytokine signaling pathways based on the resulting pattern of gene induction, which occurs selectively in genes bearing TFBMs responsive to transcription factors activated through that pathway. Analyses used aggregate indices that had been pooled across nine different technical specifications involving variations of promoter length and TFBM match stringency.⁴⁹

Confirmation of microarray findings

To validate microarray findings, three transcripts that showed upregulation in the microarray analyses, and are known to be key genes involved in inflammation (listed under Gene Ontology annotation 'inflammatory response' or 'regulation of inflammatory response') were independently assayed by qRT-PCR using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). Genes analyzed included *CCL2*, *OLR1* and *PTGS2*. Assays for each sample were carried out in duplicate using an Applied Biosystems Prism 7000 Sequence Detection System according to the manufacturer's recommended one-step thermal cycling protocol. Threshold cycle numbers for each analyte were normalized to β -actin before analysis.

Markers of inflammation

Systemic inflammation was assessed through serum levels of CRP. A high-sensitivity, chemiluminescent assay was performed on an IMMULITE 2000 (Diagnostic

Products, Los Angeles, CA, USA). This assay has an interassay coefficient of variation of 2.2% and a lower detection threshold of 0.19 mg l^{-1} . Because values were not normally distributed, CRP was log-transformed. To model the dynamics of inflammatory signaling pathways under immune challenge, we quantified PBMC production of IL-6 following stimulation with Toll-like receptor (TLR) ligands.⁵⁰ Both IL-6 production and CRP assays were conducted on the larger sample of 53. Whole blood was drawn into Vacutainer Cell Preparation Tubes (Becton Dickinson, Oakville, ON, USA), and PBMCs were isolated through centrifugation, washed and resuspended in R10 medium (Sigma, St Louis, MO, USA), and 5×10^5 PBMCs were incubated with TLR ligands following established protocols,⁵⁰ and as detailed by Miller *et al.*²² (see Supplementary Table S3 in the online supplement; purchased from Cedarlane, Burlington, ON, USA). Cells were incubated for 24 h at 37°C in a 5% CO_2 atmosphere, after which supernatants were harvested and frozen at -80°C until analysis. The samples were later assayed in duplicate for the inflammatory cytokine IL-6 using commercially available ELISA Development kits from R&D Systems (DY206E; Minneapolis, MN, USA). These kits have detection thresholds of 5 pg ml^{-1} and intra- and inter-assay coefficients of variation $< 5\%$.

Potential confounds

We also collected information on demographic and behavioral factors that could serve as potential confounds. Demographic variables included age, gender, ethnicity and current SES. Behavioral factors included smoking status, alcohol use, exercise⁵¹ and body mass index.

Results

Sample characteristics

Low early-life SES participants who experienced low versus high maternal warmth did not differ in demographic factors of age, gender, ethnicity or current SES (all P -values > 0.1), or behavioral factors including smoking, drinking, exercise and body mass index (all P -values > 0.1) (Table 1). Among the subsample on whom gene expression microarrays were conducted, those who experienced low versus high maternal warmth also did not differ on any factors from Table 1, including age ($P=0.38$), gender ($P=0.47$), ethnicity ($P=0.46$), current SES ($P=1.0$), smoking ($P=0.41$), drinking ($P=0.70$), exercise ($P=0.42$) or body mass index ($P=0.56$).

In addition, the subsample on whom gene expression microarrays were conducted was similar to the larger sample (no differences between those who were in the microarray substudy and those who were not on age ($P=0.93$), gender ($P=0.48$), ethnicity ($P=0.29$) and current SES ($P=0.82$)).

Transcriptional dynamics

A total of 491 transcripts showed a 1.20-fold or larger difference in expression between low early-life SES

Table 1 Descriptive information

	Low warmth			High warmth			P
	%	<i>M</i>	<i>s.d.</i>	%	<i>M</i>	<i>s.d.</i>	
Age		34.44	4.58		32.42	5.39	0.15
Gender (% female)	63			65			0.85
Ethnicity (% Caucasian)	63			58			0.70
Current SES (1–9 scale)		4.30	2.14		5.04	1.95	0.20
Smokers (%)	18			19			0.95
Alcohol (drinks per week)		2.80	6.23		3.06	4.94	0.87
Exercise (h per week)		3.10	4.28		5.10	8.24	0.27
Body mass index		25.55	4.39		23.73	3.88	0.12

Note: *P*-value indicates significance of differences between low- and high-warmth groups.

participants experiencing high versus low maternal warmth (corresponding to a false discovery rate of 5% (Cole *et al.*⁴⁸). Supplementary Tables S1 and S2 in the online supplement list these genes. Of the genes that were differentially expressed, 330 were upregulated and 161 were downregulated in participants who grew up with high maternal warmth.

Primary hypothesis

Results of promoter-based bioinformatics analysis indicated that there was significant downregulation of genes bearing response elements for the transcription factor NF- κ B (average 0.61-fold difference in V\$NFKAPPAB65_01 prevalence, *s.e.* = 0.11, *P* = 0.02) in low early-life SES participants who experienced high maternal warmth compared to those who experienced low maternal warmth (Figure 1 and Table 2).

Secondary analyses

Our secondary hypothesis was that in addition to NF- κ B, differences in the activity of immune activating transcription factors such as AP-1 would also be evident between the two groups. Results revealed that low early-life SES individuals who experienced high maternal warmth displayed downregulated expression of genes bearing TFBSs for AP-1 compared to those who experienced low maternal warmth (average 0.56-fold difference in V\$AP1_Q4 prevalence, *s.e.* = 0.11, *P* = 0.01) (see Figure 1 and Table 2).

Exploratory analyses

In addition to the primary hypotheses above, we performed exploratory bioinformatics analyses to evaluate whether maternal warmth among low early-life SES individuals was associated with altered activity of any other transcription-control pathways. Participants high in maternal warmth showed a relative upregulation of genes responsive to the CREB pathway (average 4.43-fold difference in V\$CREBP1_Q2 prevalence, *s.e.* = 1.01, *P* = 0.0001). They also showed a relative downregulation of genes bearing TFBSs for the GATA3 transcription factor (average 0.87-fold difference in V\$GATA3_01

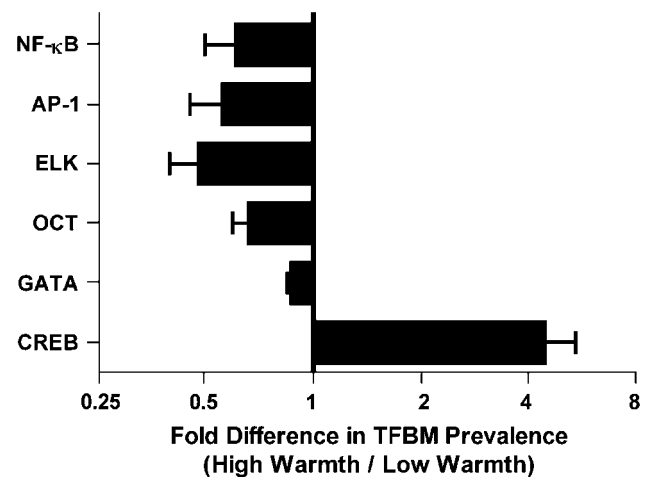


Figure 1 Fold differences in the prevalence of TFBSs for NF- κ B, AP-1, OCT, ELK, GATA and CREB in promoters of genes differentially expressed among individuals from low early-life SES backgrounds who experienced either low or high levels of childhood maternal warmth. For example, for NF- κ B, high-warmth individuals exhibited a 0.61-fold difference, or a 39% decrease, in TFBS prevalence compared to high-warmth individuals. High-warmth individuals also exhibited a 44% decrease in AP-1, a 52% decrease in ELK, a 34% decrease in OCT and a 13% decrease in GATA TFBS prevalence, along with a 4.4-fold increase in CREB TFBS prevalence, compared to low-warmth individuals.

prevalence, *s.e.* = 0.02, *P* = 0.001), OCT family factors (average 0.66-fold difference in V\$OCT1_06 prevalence, *s.e.* = 0.06, *P* = 0.005) and the ELK1 transcription factor (average 0.48-fold difference in V\$ELK1_01 prevalence, *s.e.* = 0.08, *P* = 0.006) (see Figure 1 and Table 2).

In contrast, there were no differences between maternal warmth groups on genes bearing TFBSs for the glucocorticoid receptor (GR; average 0.89-fold difference in V\$GR_Q6 prevalence, *s.e.* = 0.11, *P* = 0.36).

To identify common functional characteristics of differentially expressed genes, we conducted additional exploratory Gene Ontology analyses using

Table 2 Unadjusted and adjusted fold differences in prevalence of transcription factor-binding motifs (high-relative to low-maternal-warmth groups)

	<i>Unadjusted</i>		<i>Adjusted</i>	
	<i>Fold difference</i>	<i>P</i>	<i>Fold difference</i>	<i>P</i>
NF- κ B	0.61	0.02	0.62	0.001
AP-1	0.56	0.01	0.68	0.02
CREB	4.43	0.0001	3.28	0.001
GATA3	0.87	0.001	0.86	0.01
OCT	0.66	0.005	0.72	0.002
ELK1	0.48	0.006	0.60	0.02

Note: Adjusted analyses control for age, gender, ethnicity, current socioeconomic status, exercise, alcohol intake, body mass index and leukocyte subset distributions.

GOstat (<http://gostat.wehi.edu.au>). Gene Ontology categories overrepresented among genes upregulated in low warmth individuals included innate immune response (GO:0045087), cytokine production (GO:0001816), I κ B kinase/NF- κ B cascade (GO:0007249) and chemotaxis (GO:0006935). Functional characteristics of genes upregulated in high warmth individuals included cell development (GO:0048468), transcription activator activity (GO:0016563) and intracellular signaling cascade (GO:0007242). These patterns mirror the results of the transcription control pathway analyses in suggesting that high-maternal-warmth buffers the activation of genes involved in inflammation and immunologic activation.

Confirmatory RT-PCR

To verify results of the microarray expression analysis, we used real-time RT-PCR (qRT-PCR) to assess relative quantities of mRNA for three genes identified as up- or downregulated in low SES individuals from high warmth families. The results were concordant in all three cases; among individuals with high maternal warmth, there was a relative upregulation of *CCL2* and *OLR1*, and a relative downregulation of *PTGS2* (all *P*-values < 0.0001) (see Supplementary Figure S1 in the online supplement).

Confounds

To ensure that variation in the distribution of leukocyte subsets within the PBMC pool did not contribute to the observed disparities, we performed complete blood counts on participants' whole blood. Gene expression profiles were statistically adjusted to control for individual differences in leukocyte subset distributions. Downregulation of NF- κ B in high-maternal-warmth participants remained statistically significant in analyses controlling for leukocyte subset distributions (*P* = 0.001), as did downregulation of AP-1, GATA, OCT and ELK, and upregulation of CREB (all *P*-values < 0.05).

We next considered the possibility that demographic or behavioral factors might account for the above differences. Gene expression profiles were

statistically adjusted to control for age, gender, ethnicity, current SES, exercise, alcohol intake and body mass index. Downregulation of NF- κ B in high-maternal-warmth participants remained statistically significant (*P* = 0.002), as did downregulation of AP-1, GATA, OCT and ELK, and upregulation of CREB (all *P*-values < 0.05) (see Table 2).

Immune activation

To evaluate whether the leukocyte transcriptional disparities identified above gave rise to differential systemic immune activation, we assessed serum levels of the inflammatory biomarker CRP and PBMC production of the inflammatory cytokine IL-6 following *ex vivo* stimulation with ligands to TLRs on the larger sample. Low early-life SES individuals who experienced high maternal warmth showed directionally lower levels of CRP (log-transformed *M* = -0.31, s.d. = 0.44) compared to low early-life SES individuals who experienced low maternal warmth (*M* = -0.05, s.d. = 0.66), but these differences were not statistically significant (*t* = 1.62, *P* = 0.11). In addition, their PBMCs responded differently to TLR stimulation. High-maternal-warmth individuals produced significantly less IL-6 in response to TLR2/TLR6 stimulation with the ligand Zymosan than did those with low maternal warmth (*t* = 2.04, *P* < 0.05). PBMCs from high-maternal-warmth individuals also produced significantly less IL-6 in response to TLR9 stimulation with ODN (*t* = 2.26, *P* < 0.05). No differences were found for other ligands (see Figure 2).

Discussion

We found that in a sample of adults who grew up in low SES homes, maternal warmth during childhood was associated with reduced pro-inflammatory signaling. As adults, those who experienced high maternal warmth showed reduced immune cell responsiveness to challenge (as indicated by less PBMC-stimulated production of IL-6), and at the functional genomic level, reduced bioinformatic indications of activity by pro-inflammatory (NF- κ B) and immune-activating (AP-1) transcription factors compared to their low-warmth counterparts. These findings are significant because they suggest the possibility that the excess inflammatory activity linked to low SES may be mitigated, in part, by the presence of a warm and supportive family climate early in life.

These results are consistent with the notion that adverse social environments engender heightened signaling of pro-inflammatory transcription control pathways in childhood and adulthood,^{19–23} as well as heightened systemic inflammation in adulthood,^{52,53} and that maternal warmth experienced during childhood may serve to reduce these states. Our results add to recent theoretical discussions about how positive psychological and support factors may be able to biologically protect those who experience adversity from poor health outcomes,^{35,54,55} and complement

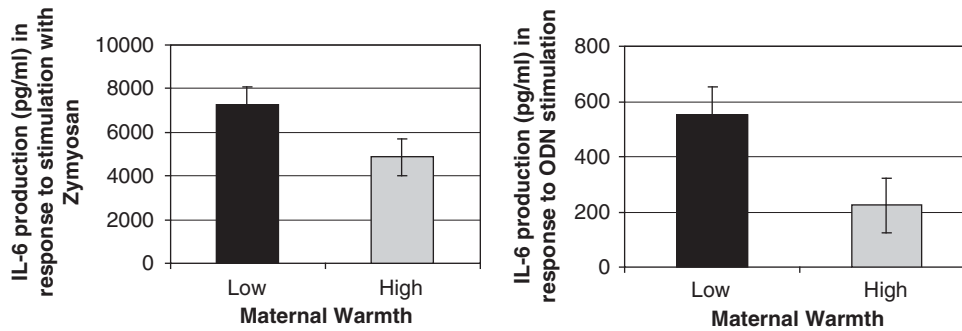


Figure 2 Differences in IL-6 production in response to TLR2/TLR6 stimulation by Zymosan and in response to TLR9 stimulation by ODN for individuals from low early-life SES backgrounds who experienced either low or high levels of childhood maternal warmth.

research that has shown protective effects of biological traits such as genetic polymorphisms under certain adverse conditions.^{56,57} Furthermore, given the causal role that inflammatory cytokines are hypothesized to have in major depression,^{29,30} the reduced inflammatory signaling seen among high-maternal-warmth individuals suggests the possibility that supportive family environments early in life could potentially be protective against later depression in part because of their effects on inflammatory pathways.

Previous human research on the biological profiles associated with positive psychosocial factors experienced under adversity has largely focused on neuroendocrine or cardiovascular risk outcomes. For example, a program to teach parenting skills to foster parents produced greater declines in cortisol among maltreated children in comparison to children in foster care whose parents did not undergo the program.⁵⁸ Similarly, maltreated children who have high ego resilience, as well as psychologically healthy adult survivors of child abuse, show different profiles of cortisol and functioning of the hypothalamic pituitary adrenal axis compared to those without resilience characteristics.^{59,60} Positive relationships with others also have been linked to less detrimental neuroendocrine and cardiovascular outcomes. For example, effects of current cumulative adversity on allostatic load (a composite of cardiovascular and neuroendocrine measures) did not occur in children who experienced high maternal warmth.³⁶ Adults who experience positive relationships currently did not show detrimental effects of low maternal warmth on adult allostatic load.³⁷ In addition, high-quality family or social relationships mitigated the relationship between low SES or parental loss and cardiovascular risk (for example, blood pressure, cholesterol profiles) in adults.^{61,62} Finally, the relationship between current SES and glycosylated hemoglobin in adults has been found to be stronger among those currently low in psychological well-being.³⁸ This study is unique both in focusing on early-life factors and their links with adult biology, as well as in showing a biological signature associated with maternal warmth that spans from systemic inflammatory

markers down to indicators of functional genomic activity.

In addition to our hypotheses relating to NF- κ B and AP-1, exploratory analyses also revealed downregulation of the OCT, ELK and GATA family of transcription factors, as well as upregulation of CREB. Interestingly, these same pathways have emerged in several other studies of the transcription control pathways linked to social adversity.^{19,22,23,63} CREB is activated by a variety of different physiological receptor systems, some of which would not be consistent with the other patterns seen in this study. However, one CREB modulator that may be relevant to the present findings is the neurotrophin brain-derived neurotrophic factor (which in addition to being found in the brain, can also be produced by immune cells⁶⁴). Low levels of brain-derived neurotrophic factor have been implicated in depression, and conversely, antidepressant treatments (both pharmacological and psychotherapeutic) have been found to increase brain-derived neurotrophic factor.^{65,66} Moreover, increases in brain-derived neurotrophic factor are linked to activation of CREB, and increases in CREB phosphorylation are also seen with antidepressant treatment.^{67,68} This suggests the possibility that the upregulation of CREB seen in this study could have implications for protection against depression. In addition, activated CREB can inhibit NF- κ B-mediated transcription by competing for transcriptional cofactors such as p300/CBP.⁶⁹ Activated CREB may reflect increased upstream activity of PKA, which can also inhibit NF- κ B.⁷⁰ Given the inhibitory effects of PKA/CREB on NF- κ B, the CREB activation observed in this study may be one plausible mechanism for reciprocal reductions in NF- κ B-mediated gene expression.

In this study, we did not find evidence that impaired glucocorticoid signaling had a role in the differential pro-inflammatory activity of low- versus high-maternal-warmth subjects (there were no significant differences in the expression of genes with response elements for the GR). This is intriguing because previous studies have suggested that the effects of adverse social environments on pro-inflammatory signaling pathways may operate in part by increased glucocorticoid resistance.^{19,20,22} Patterns for

CREB were also different, and taken together, these findings suggest that although adverse social environments and protective support factors both appear to have effects at the genomic level, each has its own specific mediational fingerprint. That is, low SES appears to increase pro-inflammatory transcription control pathways by increased glucocorticoid resistance, whereas maternal warmth appears to reduce pro-inflammatory transcription signaling by other pathways that do not involve the GR.

The present analyses focus on *cis*-regulatory activity, and thus cannot definitively rule out the possibility that altered GR signaling might contribute to the observed NF- κ B inhibition through nontranscriptional mechanisms (for example, protein–protein interactions).⁷¹ However, GR transcriptional regulation and protein–protein interactions tend to correlate because they are both induced by glucocorticoid ligation of the GR.⁷¹ In the absence of any detectable change in GR-mediated transcription, the most parsimonious interpretation of the data is that GR signaling alterations are not driving the observed inhibition of NF- κ B.

Despite differences in immune cell responsivity to challenge and bioinformatic indications of pro-inflammatory signaling, we found no statistically significant differences by maternal warmth in CRP levels, although group means were in the hypothesized direction. Because this was a healthy and relatively young sample, this could mean that differences at the level of systemic inflammation take time to emerge. Alternatively, it could be that larger samples are required to detect differences at this level.

Controlling for a variety of demographic and behavioral factors did not substantially affect study results. This indicates that the differences between low- and high-maternal-warmth participants cannot be attributed to differences in gender, age, ethnicity or various health behaviors. In addition, because the low- and high-maternal-warmth groups did not differ on current SES, the results observed in this study do not appear to be due to effects of current socioeconomic environments on inflammatory signaling pathways.

Strengths of this study include the empirical testing of theoretical models of biology profiles associated with maternal warmth using a well-defined molecular mechanism for disease risk. A second strength lies in the in-depth assessment of pro-inflammatory profiles ranging from indicators of systemic inflammation to genome-wide microarray analyses of gene expression. A third strength lies in the confirmation of early-life SES status from parents, rather than from the study participants themselves. Limitations include the small sample size (although it is similar to other microarray studies of social adversity^{19,21,23}), the retrospective nature of assessments of parental warmth and early-life SES and the inability to infer causality based on the study design. Future studies should follow clinical outcomes over time to char-

acterize the implications of these immune and gene expression profiles for psychiatric illnesses, and as well to determine the degree to which maternal warmth early in childhood protects low SES individuals, much as higher SES status does. In addition, the potential benefits of paternal, in addition to maternal, warmth would be important to establish. Finally, it would be important to identify the underlying biological mechanisms by which early-life factors contribute to persistent effects on inflammatory signaling by exploring the role of processes such as epigenetics.⁷²

In sum, this study found that high maternal warmth among those low in SES early in life was associated with reduced PBMC response to TLR stimulation, and at the genomic level, reduced bioinformatic indications of pro-inflammatory transcription factor activity. Given that pro-inflammatory states have been strongly implicated in both mental health disorders such as depression,²⁹ as well as in physical illnesses such as cardiovascular disease,²⁶ this suggests that individuals who grow up low in SES but who have warm maternal figures may be less likely to develop a number of mental and physical illnesses later in life. If true, these findings could have important public policy and public health implications. Working to alleviate poverty, as lofty and important a goal as this is, has remained an intractable problem in our society. Complementing this effort, encouraging and teaching parenting behaviors that facilitate warm emotional climates, even in the face of adversity, might prove to be a supporting, effective target of intervention (as suggested by cross-fostering and environmental manipulation studies in previous animal research^{40,73}). To the extent that promoting such environments early in children's lives can be shown to have causal protective effects, we may one day be able to partially ameliorate the inflammatory and subsequent health effects of low early-life SES environments through increasing the warmth and supportiveness of family relationships in at-risk children.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by NIH Grants HD058502, CA116778, and AG107265; BC Ministry of Child and Family Development through the Human Early Learning Partnership; and the Allergy, Genes, & Environment Research Network.

References

- 1 Dohrenwend BP, Levav I, ShROUT PE, Schwartz S, Naveh G, Link BG *et al*. Socioeconomic status and psychiatric disorders: the causation-selection issue. *Science* 1992; **255**: 946–952.
- 2 Johnson JG, Cohen P, Dohrenwend BP, Link BG, Brook JS. A longitudinal investigation of social causation and social selection

- processes involved in the association status and psychiatric disorders. *J Abnorm Psychol* 1999; **108**: 490–499.
- 3 Adler NE, Boyce T, Chesney MA, Cohen S, Folkman S, Kahn RL *et al*. Socioeconomic status and health: the challenge of the gradient. *Am Psychol* 1994; **49**: 15–24.
 - 4 Marmot MG, Shipley MJ, Rose G. Inequalities in death-specific explanations of a general pattern? *Lancet* 1984; **1**: 1003–1006.
 - 5 Chen E, Matthews KA, Boyce WT. Socioeconomic differences in children's health: how and why do these relationships change with age? *Psychol Bull* 2002; **128**: 295–329.
 - 6 House JS, Kessler RC, Herzog AR. Age, socioeconomic status, and health. *Milbank Q* 1990; **68**: 383–411.
 - 7 Adler NE, Boyce WT, Chesney MA, Folkman S, Syme SL. Socioeconomic inequalities in health: no easy solution. 1993; **269**: 3140–3145.
 - 8 Masten AS, Garmezy N, Tellegen A, Pellegrini DS, Larkin K, Larsen A. Competence and stress in school children: the moderating effects of individual and family qualities. *J Child Psychiatry* 1988; **29**: 745–764.
 - 9 Masten AS. Ordinary magic: resilience processes in development. *Am Psychol* 2001; **56**: 227–238.
 - 10 Masten AS, Obradovic J. Competence and resilience in development. *Ann N Y Acad Sci* 2006; **1094**: 13–27.
 - 11 Luthar SS, Sawyer JA, Brown PJ. Conceptual issues in studies of resilience: past, present, and future research. *Ann N Y Acad Sci* 2006; **1094**: 105–115.
 - 12 Masten AS, Shaffer A. How families matter in child development: reflections from research on risk and resilience. In: Clarke-Stewart A, Dunn J (eds). *Families Count: Effects on Child and Adolescent Development*. Cambridge University Press: New York, NY, 2006; 5–25.
 - 13 Repetti RL, Taylor SE, Seeman T. Risky families: family social environments and the mental and physical health of offspring. *Psychol Bull* 2002; **128**: 330–366.
 - 14 Chen E, Miller GE. Social context as an individual difference in psychoneuroimmunology. In: Irwin MR (ed). *Psychoneuroimmunology*, 4th edn. Elsevier: Boston, MA, 2007; 497–508.
 - 15 Panagiotakos DB, Pitsavos C, Manios Y, Polychronopoulos E, Chrysohou CA, Stefanadis C. Socio-economic status in relation to risk factors associated with cardiovascular disease, in healthy individuals from the ATTICA study. *Eur J Cardiovasc Prev Rehabil* 2005; **12**: 68–74.
 - 16 Hemingway H, Shipley M, Mullen MJ, Kumari M, Brunner E, Taylor M *et al*. Social and psychosocial influences on inflammatory markers and vascular function in civil servants (The Whitehall II study). *Am J Cardiol* 2003; **92**: 984–987.
 - 17 Chen E, Fisher Jr EB, Bacharier LB, Strunk RC. Socioeconomic status, stress, and immune markers in adolescents with asthma. *Psychosom Med* 2003; **65**: 984–992.
 - 18 Chen E, Hanson MD, Paterson LQ, Griffin MJ, Walker HA, Miller GE. Socioeconomic status and inflammatory processes in childhood asthma: the role of psychological stress. *J Allergy Clin Immunol* 2006; **117**: 1014–1020.
 - 19 Cole SW, Hawkey LC, Arevalo JM, Sung CY, Rose RM, Cacioppo JT. Social regulation of gene expression in human leukocytes. *Genome Biol* 2007; **8**: R189.
 - 20 Miller GE, Chen E, Sze J, Marin T, Arevalo JM, Doll R *et al*. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. *Biol Psychiatry* 2008; **64**: 266–272.
 - 21 Chen E, Miller GE, Walker HA, Arevalo JM, Sung CY, Cole SW. Genome-wide transcriptional profiling linked to social class in asthma. *Thorax* 2009; **64**: 38–43.
 - 22 Miller GE, Chen E, Fok AK, Walker H, Lim A, Nicholls EF *et al*. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proc Natl Acad Sci USA* 2009; **106**: 14716–14721.
 - 23 Lutgendorf SK, DeGeest K, Sung CY, Arevalo JM, Penedo F, Lucci 3rd J *et al*. Depression, social support, and beta-adrenergic transcription control in human ovarian cancer. *Brain Behav Immun* 2009; **23**: 176–183.
 - 24 Miller GE, Stetler CA, Carney RM, Freedland KE, Banks WA. Clinical depression and inflammatory risk markers for coronary heart disease. *Am J Cardiol* 2002; **90**: 1279–1283.
 - 25 Lesperance F, Frasere-Smith N, Theroux P, Irwin M. The association between major depression and levels of soluble intercellular adhesion molecule 1, interleukin-6, and C-reactive protein in patients with recent acute coronary syndromes. *Am J Psychiatry* 2004; **161**: 271–277.
 - 26 Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001; **104**: 365–372.
 - 27 Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med* 1998; **128**: 127–137.
 - 28 Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
 - 29 Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009; **65**: 732–741.
 - 30 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27**: 24–31.
 - 31 Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008; **9**: 46–56.
 - 32 Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS *et al*. Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med* 2001; **344**: 961–966.
 - 33 Yehuda R, Flory JD, Southwick S, Charney DS. Developing an agenda for translational studies of resilience and vulnerability following trauma exposure. *Ann N Y Acad Sci* 2006; **1071**: 379–396.
 - 34 Gunnar M, Quevedo K. The neurobiology of stress and development. *Annu Rev Psychol* 2007; **58**: 145–173.
 - 35 Curtis WJ, Cicchetti D. Moving research on resilience into the 21st century: theoretical and methodological considerations in examining the biological contributors to resilience. *Dev Psychopathol* 2003; **15**: 773–810.
 - 36 Evans GW, Kim P, Ting AH, Tessler HB, Shannis D. Cumulative risk, maternal responsiveness, and allostatic load among young adolescents. *Dev Psychol* 2007; **43**: 341–351.
 - 37 Singer B, Ryff CD. Hierarchies of life histories and associated health risks. *Ann N Y Acad Sci* 1999; **896**: 96–115.
 - 38 Tsenkova VK, Love GD, Singer BH, Ryff CD. Socioeconomic status and psychological well-being predict cross-time change in glycosylated hemoglobin in older women without diabetes. *Psychosom Med* 2007; **69**: 777–784.
 - 39 Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A *et al*. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997; **277**: 1659–1662.
 - 40 Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* 1999; **286**: 1155–1158.
 - 41 Levine S. Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* 2005; **30**: 939–946.
 - 42 Office for National Statistics. *The National Statistics Socio-Economic Classification*. Palgrave MacMillan: Hampshire, England, 2005.
 - 43 Krieger N, Williams DR, Moss NE. Measuring social class in US public health research: concepts, methodologies, and guidelines. *Annu Rev Public Health* 1997; **18**: 341–378.
 - 44 Parker G. Parental characteristics in relation to depressive disorders. *Br J Psychiatry* 1979; **134**: 138–147.
 - 45 Parker G. The parental bonding instrument. A decade of research. *Soc Psychiatry Psychiatr Epidemiol* 1990; **25**: 281–282.
 - 46 Luecken LJ. Parental caring and loss during childhood and adult cortisol responses to stress. *Psychol Health* 2000; **15**: 841–851.
 - 47 Tyrka AR, Wier L, Price LH, Ross N, Anderson GM, Wilkinson CW *et al*. Childhood parental loss and adult hypothalamic-pituitary-adrenal function. *Biol Psychiatry* 2008; **63**: 1147–1154.
 - 48 Cole SW, Galic Z, Zack JA. Controlling false negative errors in microarray differential expression analysis: a PRIM approach. *Bioinformatics* 2003; **19**: 1808–1816.

- 49 Cole SW, Yan W, Galic Z, Arevalo J, Zack JA. Expression-based monitoring of transcription factor activity: the TELiS database. *Bioinformatics* 2005; **21**: 803–810.
- 50 Hirschfeld AF, Bettinger JA, Victor RE, Davidson DJ, Currie AJ, Ansermino JM *et al*. Prevalence of Toll-like receptor signalling defects in apparently healthy children who developed invasive pneumococcal infection. *Clin Immunol* 2007; **122**: 271–278.
- 51 Paffenbarger RS, Blair SN, Lee IM, Hyde RT. Measurement of physical activity to assess health effects in free-living populations. *Med Sci Sports Exerc* 1993; **25**: 60–70.
- 52 Taylor SE, Lehman BJ, Kiefe CI, Seeman TE. Relationship of early life stress and psychological functioning to adult C-reactive protein in the Coronary Artery Risk Development in Young Adults Study. *Biol Psychiatry* 2006; **60**: 819–824.
- 53 Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci USA* 2007; **104**: 1319–1324.
- 54 Luthar SS, Brown PJ. Maximizing resilience through diverse levels of inquiry: prevailing paradigms, possibilities, and priorities for the future. *Dev Psychopathol* 2007; **19**: 931–955.
- 55 Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* 2009; **10**: 446–457.
- 56 Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW *et al*. Role of genotype in the cycle of violence in maltreated children. *Science* 2002; **297**: 851–854.
- 57 Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H *et al*. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; **301**: 386–389.
- 58 Fisher PA, Gunnar MR, Chamberlain P, Reid JB. Preventive intervention for maltreated preschool children: impact on children's behavior, neuroendocrine activity, and foster parent functioning. *J Am Acad Child Adolesc Psychiatry* 2000; **39**: 1356–1364.
- 59 Cicchetti D, Rogosch FA. Personality, adrenal steroid hormones, and resilience in maltreated children: a multilevel perspective. *Dev Psychopathol* 2007; **19**: 787–809.
- 60 Heim C, Newport DJ, Bonsall R, Miller AH, Nemeroff CB. Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *Am J Psychiatry* 2001; **158**: 575–581.
- 61 Luecken LJ, Rodriguez AP, Appelhans BM. Cardiovascular stress responses in young adulthood associated with family-of-origin relationship experiences. *Psychosom Med* 2005; **67**: 514–521.
- 62 Vitaliano PP, Scanlan JM, Zhang JP, Savage MV, Brummett B, Barefoot J *et al*. Are the salutogenic effects of social supports modified by income? A test of an 'added value hypothesis'. *Health Psychol* 2001; **20**: 155–165.
- 63 Cole SW, Arevalo JMG, Takahashi R, Sloan EK, Lutgendorf SK, Sood AK *et al*. Computational identification of gene-social environment interaction at the human IL6 locus. *Proc Natl Acad Sci USA* 2010; **107**: 5681–5686.
- 64 Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE *et al*. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor *in vitro* and in inflammatory brain lesions: a neuroprotective role of inflammation? *J Exp Med* 1999; **189**: 865–870.
- 65 Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 2006; **59**: 1116–1127.
- 66 Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry* 2001; **50**: 260–265.
- 67 Koch JM, Hinze-Selch D, Stingele K, Huchzermeier C, Goder R, Seeck-Hirschner M *et al*. Changes in CREB phosphorylation and BDNF plasma levels during psychotherapy of depression. *Psychother Psychosom* 2009; **78**: 187–192.
- 68 Koch JM, Kell S, Hinze-Selch D, Aldenhoff JB. Changes in CREB-phosphorylation during recovery from major depression. *J Psychiatr Res* 2002; **36**: 369–375.
- 69 Parry GC, Mackman N. Role of cyclic AMP response element-binding protein in cyclic AMP inhibition of NF-kappaB-mediated transcription. *J Immunol* 1997; **159**: 5450–5456.
- 70 Takahashi N, Tetsuka T, Uranishi H, Okamoto T. Inhibition of the NF-kappaB transcriptional activity by protein kinase A. *Eur J Biochem* 2002; **269**: 4559–4565.
- 71 Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med* 2005; **353**: 1711–1723.
- 72 Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR *et al*. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847–854.
- 73 Champagne FA, Meaney MJ. Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. *Behav Neurosci* 2007; **121**: 1353–1363.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)