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Prenatal Maternal Depression and Neonatal Immune Responses

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Abstract

Objective: The aim of the study was to examine the association of lifetime maternal depression with regulation of immune responses in the infant, measured by cytokine levels and lymphocyte proliferation in cord blood mononuclear cells (CBMC) collected at delivery.

Methods: We studied women recruited in early pregnancy into the Project Viva longitudinal cohort who had cord blood assayed after delivery (N = 463). Women reported about depressive symptoms in mid-pregnancy (Edinburgh Postnatal Depression Scale) and depression history by questionnaire. Immune responses were assayed by an index of lymphocyte proliferation (LP), and concentrations of five cytokines (IL-6, IL-10, IL-13, TNF- α , and IFN- γ) after incubation of CBMC either in medium alone or stimulated with phytohemagglutinin (PHA), cockroach extract (Bla g 2), or house dust mite extract (Der f 1). We examined associations of maternal depression with these sets of cytokine measures using multivariable linear or tobit regression analyses.

Results: After adjustment for confounders (mother's age, race/ethnicity, and education, household income, season of birth, and child sex), levels of IL-10 after stimulation with cockroach or dust mite allergen were lower in cord blood from ever versus never depressed women, and a similar trend was evident in IL-10 stimulated with PHA (% difference: Bla g 2 -41.4, $p=0.027$; Der f 1 -36.0, $p=0.071$; PHA -24.2, $p=0.333$). No significant differences were seen in levels of other cytokines or LP.

Conclusions: Maternal depression is associated with offspring immune responses at birth, which may have implications for later life atopic risk or immune function.

Keywords

maternal prenatal depression; cord blood mononuclear cells; cytokines; intergenerational effects

Introduction

Depression during pregnancy is relatively common, with prevalence estimates ranging from 7 to 18% in the US, and similar prevalence in many low- and middle-income countries (1, 2). Beyond affecting the well-being of the mother herself, maternal depression or depressive symptoms during pregnancy have been associated with adverse birth outcomes (e.g., preterm delivery, low birth weight) (3) and with health-related outcomes in early childhood (3–5). Mechanisms by which maternal depression may lead to adverse outcomes in offspring have not yet been clearly identified, but one hypothesized pathway is through alterations in biological processes related to immune regulation. Healthy immune regulation occurs in part by a counter-balancing of pro- and anti-inflammatory effects of cytokines. Prior work has suggested that these processes are dysregulated in individuals with depressive symptoms and disorders (6–8). This dysregulation is hypothesized to result in part from over-activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to persistent secretion of glucocorticoids and other hormones that contribute to regulating inflammatory responses (6). As one result, immune cells become less sensitive to glucocorticoids' anti-inflammatory effects; this glucocorticoid insensitivity leads to chronic inflammation, which can cause physiological damage (7) and appears to play a role in the development of diseases such as atherosclerosis, diabetes, and obesity (8).

More specifically, compared to non-depressed individuals, depressed individuals may exhibit higher circulating levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), two pro-inflammatory cytokines (8). While these cytokines play an important and necessary role in responding to acute infection, in a dysregulated system they may fail to return to baseline after activation, contributing to chronic low-grade inflammation [6]. Moreover, counterbalancing anti-inflammatory cytokines, such as interleukin-10 (IL-10), may be lower in depressed compared to non-depressed individuals (8). These relationships appear to hold in pregnancy: while only one small case-control study has looked at maternal depression per se and maternal cytokines (9), studies in the related literature on maternal stress find that in pregnant women higher maternal stress is associated with higher levels of hormones released by HPA axis activation, and higher circulating levels of IL-6 and TNF- α and lower IL-10 (10, 11).

Whether maternal depression may exert an intergenerational effect on her offspring's immune response is still unclear. Studies in depressed pregnant mothers and their newborns have demonstrated changes in the infant's hormonal and neurotransmitter patterns that indicate potential dysregulation of the infant's HPA axis and related hormones and neurotransmitters (12). Few studies, however, have assessed the associations of prenatal maternal depression with offspring cytokine responses. The South London Child Development Study, a prospective, observational study of the long-term effects of offspring exposure to prenatal maternal depression, followed 103 children of mothers recruited during pregnancy from birth until age 16. This study found that levels of C-reactive protein (CRP), a pro-inflammatory protein whose production is stimulated by IL-6 and TNF- α , were elevated in young adults whose mothers were depressed during pregnancy (13). Three studies have examined whether maternal prenatal depression may lead to dysregulation of offspring cytokines earlier in life, and they have reported conflicting results: one retrospective study of 136 women recruited at birth (9) found no correlation between major depressive disorder and cord blood immune biomarkers; a small case-control study of preterm and term births (14) found positive correlations between depressive symptoms in pregnancy and cord blood levels of IL-18 (but not IL-10, IL-13, IFN γ) only in preterm births; while a prospective study of 98 pregnant women with allergic disease (15) found a global positive association between depressive symptom in pregnancy and the cytokines IL-6, IL-10, IL-13 and IFN- γ in cord blood.

Given the limitations of the existing literature, the current study sought to evaluate, using data from a longitudinal pregnancy cohort, whether maternal depression was associated with alterations of offspring immune responses evident at birth. We hypothesized that maternal depression would lead to higher levels of lymphocyte proliferation (LP) pro-inflammatory cytokines (IL-6, TNF- α) and IL-13, and lower levels of anti-inflammatory cytokines (IL-10) produced by CBMC collected at delivery. Because depression is a recurring disease (16, 17), and because, as in stress, the effects on health are considered to be the result of cumulative burden rather than acute exposure (18), we considered as our primary predictor mothers who either reported a history of depression or who reported symptoms consistent with depression during pregnancy. In addition, because increased cytokine production in response to immune stimulant exposure can be interpreted as a measure of the immune system's competence, we assessed cytokine production upon stimulation of CBMCs with three immune stimulants (19). We considered as covariates factors that might alter the risk of maternal depression and influence immune responses, including mother's demographics, pre-pregnancy body mass index (BMI, kg/m²), blood pressure, and parental history of atopy or asthma (20). We also included sex of the child (21), season of birth, and maternal smoking, since they have been shown to influence cord blood immune parameters.

Methods

Study sample

Project Viva, a longitudinal pregnancy cohort, has been described in detail elsewhere (22). Briefly, pregnant mothers were enrolled during 1999–2002 at mothers' initial prenatal obstetric visits (median 9.9 weeks of gestation) at eight locations of Atrius Harvard

Vanguard Medical Association, a multispecialty group practice in urban and suburban Eastern Massachusetts. All participants delivered at one of two area hospitals, but only one hospital was willing to facilitate cord blood collection. Women were eligible for enrollment if they were less than 22 weeks pregnant, had a singleton pregnancy, were able to answer questions in English, and had no plans to move out of the study area prior to delivery. Mothers provided written informed consent upon enrollment, including for access to medical records and cord blood collection at delivery. Delivering clinicians collected venous umbilical cord blood from non-emergent deliveries at the participating hospital (N=1029 of the 2128 live singleton births), among which we measured cytokines or LP for N=463 (see flow chart of sample sizes, Figure, Supplemental Digital Content 1).

Study staff visited women after routine appointments during the first and second trimesters, and 1–3 days after delivery on the post-partum maternity floor. The study was approved by the Institutional Review Board of Harvard Pilgrim Health Care.

Measures

Depression Assessment—We characterized women as having experienced depression if they met criteria for depression before or during pregnancy, as detailed in the following sections. The reference group was women who experienced depression neither before nor during pregnancy.

Depression during pregnancy: Mothers reported depressive symptoms during the past seven days at the mid-pregnancy visit using the Edinburgh Postnatal Depression Scale (EPDS), a 10-item questionnaire that has been validated for use in pregnancy (23). Response options are on a Likert scale ranging from 1 (most of the time) to 4 (not at all), scored so that a higher score indicates more depressive symptoms. Although various EPDS thresholds for *probable prenatal depression* have been identified (24), a cut-off score of greater than 12 is commonly used (25, 26), with prior validation of this cutoff against diagnostic clinical interviews indicating a specificity of 78% and a sensitivity of 86% (23). We categorized women who had EPDS score >12 as well as women who were prescribed antidepressants during pregnancy regardless of their EPDS score as having depression during pregnancy.

Antidepressants during pregnancy (yes/no) was determined via information drawn from each woman's electronic medical record. We compiled a list of all medications prescribed during pregnancy, and a study physician identified antidepressants.

Pre-pregnancy depression history (yes/no) was determined using three questions included on the mid-pregnancy questionnaire. Participants were asked an initial screening question: "Before this pregnancy, was there ever a period of time when you were feeling depressed or down or when you lost interest in pleasurable activities most of the day, nearly every day, for at least 2 weeks?" Women who responded affirmatively were asked to complete two follow-up questions: (1) "Before this pregnancy, did you ever see a health care professional who said that you were depressed?" and (2) "Before this pregnancy, did a health care professional ever prescribe a medication for you for depression?" We considered women who responded affirmatively to the screener question and to one or both of the follow-up questions to have a history of depression.

Ever depressed: women were characterized as ever depressed (yes/no) if they scored as having depression during pregnancy or a history of depression, or both.

Immune Outcome Assessment—The cytokine panel obtained in Project Viva includes major pro- and anti-inflammatory cytokines (TNF- α and IL-6; IL-10 respectively). In addition, molecules indicating differential T-cell stimulation were measured: interferon-gamma (IFN- γ), which promotes differentiation of the T-helper cell 1 (Th1) lineage of the adaptive immune system; and interleukin 13 (IL-13), which is produced mainly by cells in the T-helper cell 2 (Th2) lineage of the adaptive immune system. Lymphocyte proliferation (LP) serves as a global measure of immune activation.

Cord blood samples were collected by needle/syringe from the umbilical vein after delivery, were processed within 24 hours without freezing, and CBMC were isolated from umbilical cord blood by density gradient centrifugation with Ficoll-Hypaque Plus (Pharmacia, Uppsala, Sweden). Cells were washed in RPMI 1640 and diluted in 10% human serum (Biowhittaker, Walkersville, MD) to a concentration of 5×10^6 cells/ml. Culture conditions, described below, were ascertained as appropriate from dose-response and time-kinetic experiments, as previously reported (27).

Lymphocyte proliferation: 4 replicates of 0.5×10^6 CBMC/well were incubated in 96-well round-bottom tissue culture plates (Corning, New York, NY) at 37°C in 5% CO₂, either unstimulated (incubated in media alone), or stimulated with one of three different immune system stimulants (see below), and cultured for 72 hours. CBMC were then pulsed with 1 μ Ci [3H]thymidine for an additional 8 hours. Cultures were maintained at 37°C in a humidified 5% CO₂ incubation chamber. Cells were harvested with a Tomcat Mach II harvester (Wallac, Turku, Finland) onto filter plates, which were read in a β -counter. Proliferation was quantified by stimulation index (SI), calculated as the ratio of mean counts per minute of stimulated to unstimulated replicates (28).

Cytokines: Aliquots of 5.0×10^6 CBMC were incubated in triplicate as described above. Cell supernatants were harvested after 72 hours and analyzed for production of the cytokines IFN- γ , IL-13, IL-6, IL-10, and TNF- α using enzyme-linked immunosorbent assay (Endogen, Rockford, IL) according to the manufacturer's protocol. Sensitivities of the assays were <2 pg/mL for IFN- γ , <7 pg/mL for IL-13, <3 pg/mL for IL-10, and <2 pg/mL for TNF- α . All IL-6 samples were detectable. The % of samples below the detection limit varied from 0 to 72.3% (see Table, Supplemental Digital Content 2).

Stimulants: Cell aliquots were stimulated with one of the following: 5 μ g/mL phytohemagglutinin (PHA), 30 μ g/mL cockroach extract (Bla g 2), or 30 μ g/mL house dust mite extract (Der f 1). PHA is a mitogen (29, 30) with response not dependent on antigen-presenting cells. Bla g 2 and Der f 1 are two common environmental allergens: prior work has demonstrated that CBMC respond to these extracts by proliferating and secreting cytokines (31, 32). Such activation is commonly interpreted as a measure of the competence of the adaptive immune system to react to antigen (19). Current understanding of the dynamics of the neonate's immune system is too limited to identify a specific level of response as "more" or "less" healthy; instead, we seek to determine whether responses differ

in offspring of ever depressed mothers compared to mothers who have never experienced depression.

Covariate Assessment—At enrollment, women reported their age, race/ethnicity, marital status, household income, smoking history, history of hypertension, and maternal and paternal history of asthma and atopy. Mothers self-reported pre-pregnancy weight and height, from which we calculated body mass index (BMI, kg/m²). We obtained child sex and date of birth from the hospital delivery record. Information on cigarette use during pregnancy was collected by questionnaire (early- and mid-pregnancy) and interview (delivery), and categorized as never/former/smoked during pregnancy. To ascertain the presence of hypertensive disorders of pregnancy, we evaluated prenatal records for blood pressure and urine protein results. We created a 4-level variable with the categories normotensive, gestational hypertension, pre-eclampsia, and chronic hypertension in line with guidelines from the 2000 National High Blood Pressure Working Group on High Blood Pressure in Pregnancy (33).

Statistical Analysis

To address non-normality of the cytokine and lymphocyte distributions, we log-transformed them. We used Chi-square, independent T, or ANOVA tests to compare covariate distribution across women who were ever versus never depressed.

We used separate multivariate regression models to assess relationships of maternal depression with each outcome, namely LP, IL-10, IFN- γ , TNF- α , IL-6, and IL-13, each upon stimulation with PHA, B16.8 or Der f 1 extract and upon incubation without stimulant (MED). Our primary exposure was lifetime depression (ever depressed, yes/no). We also ran separate models for each dichotomous depression measure (depression during pregnancy, depression history, antidepressant prescription).

For most immune outcomes, we used linear regression models. However, for cytokines with 9% or more of samples falling below the detection level of the assays, we used tobit regression (using SAS PROC QLIM; See Table, Supplemental Digital Content 2). Tobit regression adjusts for censored data by combining a probit model to account for the fraction of samples censored (i.e. below LOD) with a truncated regression model for those outcome values that score above the censored cutoff.

We tested two separate models for each combination of depression and immune outcome. Model 1 adjusted for likely confounders (mother's age, race/ethnicity, education and household income, each categorized as reported in Table 1; as well as season of birth and child sex). Model 2 added a set of covariates that might either confound or lie on the pathway between lifetime depression and cord blood immune outcomes (20), including mother's pre-pregnancy BMI (continuous), history of hypertension and pregnancy disorders of hypertension (categorical), and smoking (categorical) (20, 34). Maternal and paternal history of atopy were not associated with any of the immune outcomes so we did not include these variables in our models.

As immune outcomes in our models were log-transformed, to increase interpretability of the regression coefficients we exponentiated the coefficients to obtain differences in the ratio of the expected geometric means of the outcome variable, and express these in the figures as the % difference in the geometric mean of the outcome variable in depressed compared to non-depressed women.

We additionally conducted two sensitivity analyses. Because previous studies have found associations between perinatal complications and cord blood cytokine concentrations (35, 36), we re-ran all models ($n = 357$) excluding women whose pregnancies resulted in a cesarean delivery, a preterm birth (defined as <34 weeks of gestation), or small-for-gestational-age (defined as $<10^{\text{th}}$ percentile of birthweight for gestational age and sex). Antidepressants may serve as an indicator of severe depression but could also have biological effects due to medication per se. To ensure that we were assessing the effect of depression rather than of depression medications, we re-ran all models ($n = 445$) excluding women who were taking antidepressants during pregnancy.

Missing data: 102 women were missing data on the primary exposure variable. To maintain sample size, reduce bias, and improve model precision we imputed depression scores and covariate values for all individuals missing these measures using a chained equation multiple imputation model (PROC MI in SAS). Imputed values were derived using the full Project Viva cohort ($n=2128$), by including exposure and outcome variables, all covariates, and other potential predictors of the outcome in the imputation model (37). We generated 50 imputed data sets, and combined them using PROC MIANALYZE (38). Final models included participants with imputed depression and covariate data. Participants missing measurements of outcome for a given exposure-outcome analysis were excluded from that particular analysis.

We performed all analyses in SAS Version 9.4 (SAS Institute Inc., Cary, NC).

Results

Participant characteristics

Women in versus excluded from the study sample showed few differences (see Table, Supplemental Digital Content 3). Compared to those excluded, women in the study sample were more likely white (71% vs. 65%) and had fewer cesarean deliveries (19% vs. 25%). In the study sample, 80 women of 361 with data on depression (22%) were ever depressed. Of these 80, 34 women had pregnancy depression without a history of depression, 27 had a history of depression without pregnancy depression, and 19 had both pregnancy depression and a history of depression.

Comparing women who were ever versus never depressed highlights several differences (see Table 1). Women who were ever depressed were slightly younger at enrollment (mean age 31.9 v. 32.5 years), and were more likely to have a household income of less than \$40,000/year (18% vs. 9%) and smoke during pregnancy (21% vs. 8%). Women who were ever depressed also had more babies born in winter and fewer in spring or fall.

Associations between depression and immune outcomes

Unadjusted Spearman correlation coefficients for log-transformed immune outcomes and EPDS score ranged from -0.06 to 0.08 (see Table, Supplemental Digital Content 4). In our models, we first looked at the anti-inflammatory cytokine, IL-10. In models adjusted for likely confounders, levels of IL-10 from unstimulated cells did not differ by ever vs. never maternal depression (Figure 1). However, after stimulation with cockroach or dust mite allergen, IL-10 levels were substantially lower in ever depressed women [% difference (95% CI): Bla g 2 -41.4 , (-63.4 , -6.1), $p=0.027$; Der f 1 -36.0 , (-60.6 , 4.0), $p=0.071$], and a similar trend was evident in IL-10 stimulated with PHA [-24.2 (-56.8 , 33.0), $p=0.333$].

Adding mother's pre-pregnancy BMI and smoking history to the model did not change the results, nor did adding pregnancy variables (See Table, Supplemental Digital Content 5).

We next looked at TNF- α , IL-6, IFN- γ , IL-13, and LP. Although there was a general trend for most stimulated markers to be lower in women who were ever versus never depressed, none of these trends reached statistical significance (Figure 2). Adding additional covariates to the base model did not change any of the findings. IL-13 had a high proportion of samples ($>50\%$) below the assay's level of detection (Table, Supplemental Digital Content 2). There was no evidence that the proportion of samples above the level of detection was associated with maternal depression (data not shown).

Additional Analyses

We separately considered each depression indicator's association with stimulated or unstimulated levels of IL-10 (but not with cytokines for which no primary associations were found). Findings were largely similar to those with the combined measure of depression (Figure 3), although confidence intervals were generally wide and crossed the null given relatively small numbers in each group. Excluding women with perinatal complications did not change results; and excluding women who had prescriptions for antidepressants during pregnancy caused a slight attenuation in effect estimates but the basic pattern of associations was unchanged (data not shown).

Discussion

In a longitudinal cohort of 463 pregnant women, mothers who reported a history of depression or depression during pregnancy had lower CBMC production of the anti-inflammatory cytokine IL-10 in response to stimulation. Neither LP nor levels of the pro-inflammatory cytokines TNF- α and IL-6 differed in children of mothers who ever (vs never) experienced depression. Although this finding was unexpected, intergenerational studies in both animals (39) and humans (40) have suggested that exposure to depression or stress *in utero* may lead to a general downregulation of cytokines and other immune processes in the offspring.

As a predominantly anti-inflammatory cytokine, IL-10 plays a central role in control of both innate and cell-mediated immunity. Thus, a decrease in levels of this cytokine may dampen the potential of the newborn to effectively down-regulate proinflammatory stimuli (41). If

effects of prenatal depression on reduction in IL-10 production persisted, they might adversely affect a child's response to immune challenges through the life course.

As yet, data are sparse concerning the relation between maternal prenatal depression, offspring cytokines, and offspring health outcomes. In contrast to our findings, a small prospective study found higher levels of CBMC IL-10 (either unstimulated or stimulated with dust mite antigen) from women classed as "mildly depressive" (Beck Depression Inventory 10) in pregnancy. Levels of pro-inflammatory cytokines and lymphocyte proliferation were higher as well. All of these women had allergic disease (15), so it is unclear how generalizable this finding might be to non-allergic women. Another study reported that children of women with prenatal depression had higher levels of the pro-inflammatory biomarker CRP (after accounting for their own depression status) when they were adults at age 25 (13). Here, cytokine outcomes were not evaluated early in life, and although the authors adjusted for mother's and child's depression, other, unmeasured factors after birth and throughout childhood may have contributed to this inflammatory response. Similar to our findings, a prospective birth cohort study recently found significant inverse associations between prenatal maternal depression and Th2 cytokines (IL-4, IL-5, and IL-13) from peripheral blood mononuclear cells collected from children at age 3, upon treatment in vitro with a variety of immune stimuli including PHA and dust mite antigen (40).

Broadly speaking, evidence is emerging for links among prenatal maternal stress or depression, alterations in maternal HPA axis and immune system functioning, and offspring HPA axis function or immune response at birth and beyond. Glucocorticoids are known to cross the placenta, and it has been suggested that fetal exposure to higher levels of maternal glucocorticoids, which might be due to either prenatal maternal stress (42) or prenatal maternal depression (43), could adversely affect fetal immune maturation (42, 44). Although details differ, maternal psychosocial stress and prenatal depression have both been linked to epigenetic changes in placental genes involved in maternal-fetal glucocorticoid transfer (45). Specific to depression, studies have consistently shown that women with versus without untreated depression or depressive symptoms in pregnancy are more likely to have newborns with higher levels of cortisol (as well as norepinephrine, a hormone released when the sympathetic nervous system is activated) (3). Recent epigenetic studies have also found evidence of differences in newborns exposed to mother's prenatal depression (12). For example, a candidate gene study found that increased maternal depressed mood in the third trimester was associated in the infant with increased methylation of a binding site on the gene NR3C1, which codes for the glucocorticoid receptor (46). Thus, the present study adds to a small but growing body of literature supporting the hypothesis that exposure to maternal prenatal depression affects offspring immune parameters at birth.

Because we conceived of depression as a recurring disease, we examined the effects on the newborn of the mother's ever having experienced depression before the birth. We also separately examined the effects of probable depression during pregnancy and of reporting a history of depression before the pregnancy, and found they were similar, supporting the conclusion that timing of overt depressive symptoms is not the critical factor in the association between maternal depression and neonate immune markers.

Another debate in the literature revolves around whether effects of maternal depression on offspring outcomes are primarily due to the impact of antidepressant medication itself, rather than the underlying depression (47). Given the similarity of our findings including or excluding women on antidepressants, it seems less likely that results are primarily due to a drug effect.

Our study has several limitations. Not all members of the cohort had cord blood drawn at delivery and assayed (Table, Supplemental Digital Content 6). Although in most respects those in the analytic sample were similar to the full cohort, they were less likely to have had a cesarean delivery (Supplemental Digital Content 3) because cord blood samples were only collected during non-emergency deliveries. Prior work has found higher concentrations of IFN- γ , TNF- α , and IL-6 in cord blood of babies born vaginally versus by cesarean section (35), which might result in finding a stronger positive association between maternal depression and cord blood levels of these cytokines in the analytic sample. However, we did not find any evidence of positive associations. Alternatively, because those with emergency cesareans were more likely excluded from the analytic sample, and perinatal complications may be on the pathway from maternal depression to offspring immune responses, we may be underestimating effects. However, after excluding any pregnancies that resulted in cesarean delivery, our results were substantially unchanged.

Depressive symptoms were measured only once during pregnancy. Exposure misclassification due to experience of depression earlier or later in pregnancy would bias results towards the null if the timing of prenatal insults on fetal development is important. There is some support for such timing effects. For example, a study of methylation status of brain-derived neurotrophic factors in neonatal cord blood found an association with maternal depressed mood in the third, but not the second trimester (48).

Although all of the cytokine levels were measurable, those that fell within the linear portion of the standard curve may have been measured with more precision than the lowest and highest values, which may make it harder to see effects. We examined patterns of association across correlated outcomes, and interpreted the results according to *a priori* hypotheses, without correcting for multiple outcomes, since doing so increases the possibility of a Type II error, and may overlook the possibility of an informative finding (49–51). However, when we controlled for the false discovery rate for IL-10 outcomes (52), we found that the p-value for IL-10 stimulated with Bla g2 remained significant (for rationale, see Supplemental Digital Content 7). In addition, although our sample size of 463 was large enough to detect a somewhat small or medium effect (53), the study may not be sufficiently powered to make a null result fully informative regarding a small effect.

The Project Viva cohort is composed of predominantly white, well-educated women and their children residing in the Boston area. Whether these results will generalize to populations with a higher proportion of lower SES or minority groups remains to be seen. However, of note is that the prevalence of depression among poor and minority groups is higher than in white/higher SES groups. Thus, if our finding that prenatal depression affects neonate immune responses is replicated in more disadvantaged populations, it may provide insight into the origins of social disparities in health.

This study has several strengths as well. Exposures were assessed before outcomes occurred; a variety of measures of prenatal depression were available; and measurements were available for a rich array of potential confounders and predictors of cord blood immune parameters.

Conclusion

Prenatal depression is relatively common, and recent randomized controlled trials have demonstrated that it is treatable (54, 55). Understanding the mechanisms by which a mother's experience of depression before the birth of her children can affect offspring health will provide insights into how children get started on a more or less healthy developmental trajectory, and potentially make more clear when and how one might buffer such effects to ensure that all children have a healthy start in life. In concert with other studies implicating the mother's HPA activity and level of inflammation in dysregulation of the fetus's own HPA and immune responses, our study found that neonates who were exposed to maternal depression had lower levels of an important anti-inflammatory cytokine at birth. The implications for future immune dysregulation or disease as the child grows are still not clear. Further longitudinal studies in this cohort (56) and others can shed light on whether immune differences observed at birth persist into childhood and beyond, and whether they have an impact on an individual's health. In addition, such studies can determine whether other alterations not yet seen at birth, such as reduced lymphocyte effector proliferation, globally diminished immune responses, or elevated inflammatory responses (13, 39, 57) become apparent later in life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Conflicts of Interest and Source of Funding:

Project Viva is supported by the National Institutes of Health (R01 HD 034568, R01AI102960). The authors have no conflicts of interests to report.

Glossary

Bla g 2	cockroach extract
BMI	body mass index
CBMC	cord blood mononuclear cells
Der f 1	house dust mite extract
EPDS	Edinburgh Postnatal Depression Scale
HPA	hypothalamic-pituitary-adrenal axis
IFN-γ	interferon-gamma

IL-6	interleukin-6
IL-10	interleukin-10
LP	lymphocyte proliferation
MED	incubated without stimulant
PHA	phytohemagglutinin
Th1	T-helper cell 1
Th2	T-helper cell 2
TNF-α	tumor necrosis factor-alpha
SI	stimulation index

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Figure 1:

Percent difference (95% CI) in concentration of IL-10 in children of ever vs. never depressed women, for unstimulated cord blood lymphocytes or for those stimulated with PHA, cockroach antigen, or dust mite antigen.

Outcomes were log transformed for analysis and exponentiated to arrive at % difference.

Unstimulated IL-10 was analyzed using tobit regression; others were analyzed using linear regression. Models adjusted for maternal age, race/ethnicity, and education; household income; and child sex and season of birth. Sample sizes: 442 (unstimulated, PHA), 368 (cockroach antigen), 362 (dust mite antigen).

Figure 2:

Percent difference (95% CI) in lymphocyte SI or cytokine concentration in children of ever vs never depressed women.

Outcomes were log transformed for analysis and exponentiated to arrive at % difference.

IFN- γ was analyzed using tobit regression; others were analyzed using linear regression.

Models adjusted for maternal age, race/ethnicity, and education; household income; and child sex and season of birth. For sample sizes for each analysis, see Figure, Supplemental Digital Content 1).

Figure 3:

Percent difference (95% CI) in concentration of IL-10 for all depression measures, from unstimulated cord blood samples or for those stimulated with PHA, cockroach antigen, or dust mite antigen.

Outcomes were log transformed for analysis and exponentiated to arrive at % difference. Unstimulated IL-10 was analyzed using tobit regression; others were analyzed using linear regression. Models adjusted for maternal age, race/ethnicity and education; household income; and child sex and season of birth. For sample sizes for each analysis, see Figure, Supplemental Digital Content 1.

Table 1. Participant characteristics according to depression status (n, % unless otherwise stated).^a

	Never depressed N=281	Ever depressed N=80
Mother		
Pre-pregnancy BMI, kg/m ² : Mean (SD)	23.9 (4.8)	24.7 (5.0)
Age, years: Mean (SD)	32.5 (4.5)	31.9 (5.6)
Race/ethnicity		
. Black	24 (9)	8 (10)
. Hispanic	12 (4)	6 (8)
. White	220 (79)	56 (70)
. Other	24 (9)	10 (13)
Education high school ^d	17 (6)	8 (10)
Household income \$40,000/year ^d	24 (9)	13 (18)
Gestational hypertension or pre-eclampsia	20 (7)	11 (14)
Pregnancy smoking status		
. Never	199 (72)	49 (61)
. Former	57 (21)	14 (18)
. Smoked during pregnancy ^b	21 (8)	17 (21)
Cesarean delivery	52 (19)	16 (20)
Child		
Season of birth ^c		
. Winter	56 (20)	29 (36)
. Spring	80 (28)	14 (18)
. Summer	78 (28)	24 (30)
. Fall	67 (24)	13 (16)
Child sex female	138 (49)	35 (44)

^aTable 1 based on non-imputed data. 102 individuals were missing data on depression.^bP < 0.001^cP < 0.01^dP < 0.05.