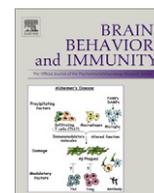




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Epstein-Barr virus reactivation during pregnancy and postpartum: Effects of race and racial discrimination

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ABSTRACT

Objective: Adverse pregnancy outcomes, including preterm birth, are markedly higher among African-Americans versus Whites. Stress-induced immune dysregulation may contribute to these effects. Epstein-Barr virus (EBV) reactivation provides a robust model for examining cellular immune competence. This study examined associations of EBV virus capsid antigen immunoglobulin G (VCA IgG) with gestational stage, race, and racial discrimination in women during pregnancy and postpartum.

Methods: Fifty-six women (38 African-American, 18 White) were included. African-Americans and Whites did not differ in age, education, income, parity, or body mass index ($ps \geq .51$). During the 1st, 2nd, and 3rd trimester and ~5 weeks postpartum, women completed measures of racial discrimination, perceived stress, anxiety, depressive symptoms and health behaviors. EBV VCA IgG antibody titers were measured via ELISA in serum collected at each visit.

Results: In the overall sample, EBV VCA IgG antibody titers were lower in the 3rd versus 1st trimester ($p = .002$). At every timepoint (1st, 2nd, 3rd trimester and postpartum), African-American women exhibited higher serum EBV VCA IgG antibody titers than Whites ($ps < .001$). This effect was most pronounced among African-Americans reporting greater racial discrimination [$p = .03$ (1st), $.04$ (2nd), $.12$ (3rd), $.06$ (postpartum)]. Associations of race and racial discrimination with EBV VCA IgG antibody titers were not accounted for by other measures of stress or health behaviors.

Conclusions: Compared to Whites, African-American women showed higher EBV VCA IgG antibody titers, indicative of impaired cellular immune competence, across pregnancy and postpartum. This effect was particularly pronounced among African-American women reporting greater racial discrimination, supporting a role for chronic stress in this association. In women overall, EBV antibody titers declined during late as compared to early pregnancy. This may be due to pregnancy-related changes in cell-mediated immune function, humoral immune function, and/or antibody transfer to the fetus in late gestation. As a possible marker of stress-induced immune dysregulation during pregnancy, the role of EBV reactivation in racial disparities in perinatal health warrants further attention.

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1. Introduction

Maternal stress during pregnancy has been associated with increased risk of adverse birth outcomes, but the mechanisms by which this occurs remain uncertain (Committee on Understanding Premature Birth and Assuring Healthy Outcomes, 2007). Stress constructs associated with risk of preterm birth include perceived stress, general distress, stressful life events, anxiety, and depressive

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symptoms (Committee on Understanding Premature Birth and Assuring Healthy Outcomes, 2007). Moreover, perceived racial discrimination has repeatedly been linked to increased risk of preterm delivery among African-American women (Rosenberg et al., 2002; Dole et al., 2003, 2004; Collins et al., 2004; Mustillo et al., 2004). Thus, the chronic stress of discriminated racial minority status has been implicated in the substantial racial disparities in preterm birth and low birth weight among African-American women versus women of other races/ethnicities in the US (Giscombe and Lobel, 2005).

Given the linkage of stress to adverse pregnancy outcomes, an emerging literature is focusing on potential biological mechanisms for these associations. In terms of immune parameters, inflammatory markers have been a primary focus. Factors including

perceived stress, stressful life events, depressive symptoms, and trauma have been associated with higher circulating inflammatory markers including interleukin (IL)-6, tumor necrosis factor (TNF)- α , and IL-1RA in pregnant women (Coussons-Read et al., 2005; Ruiz et al., 2007; Paul et al., 2008; Christian et al., 2009; Blackmore et al., 2011; Cassidy-Bushrow et al., 2012) as well as exaggerated inflammatory responses to *in vivo* and *in vitro* immune challenges (Coussons-Read et al., 2007; Christian et al., 2010). Linking such effects to birth outcomes, in a study of 173 women followed across pregnancy, an association between prenatal stress and gestational age at birth was mediated by levels of circulating inflammatory markers (Coussons-Read et al., 2012).

In addition to promoting inflammation, chronic stress can suppress cellular immune function. Viral reactivation provides a robust model for examining cellular immune competence. By adulthood, >90% of the US population is infected with Epstein-Barr virus (EBV) (Jones and Straus, 1987). Once infected, an individual carries the virus for life. The virus latently infects B-lymphocytes, which are the primary cells in which the virus is maintained. The cellular immune system plays an important role in controlling replication of the virus. However, under conditions of immunosuppression, the virus may reactivate causing a release of viral proteins and activating a humoral immune response as indexed by higher EBV VCA IgG antibody titers (Glaser and Kiecolt-Glaser, 1994).

Although EBV reactivation generally causes few to no symptoms in healthy individuals, it can serve as a marker of stress-induced immune dysregulation (Glaser and Kiecolt-Glaser, 2005). For example, EBV VCA IgG antibody titers are elevated among older adults experiencing the chronic stress of caregiving for a spouse with dementia versus well-matched controls (Kiecolt-Glaser et al., 1991). EBV reactivation is also responsive to transient stressors; medical students as well as military academy students exhibit higher EBV VCA IgG antibody titers during major academic exams compared to several weeks before or after exams (Glaser et al., 1985, 1999). Other research has shown that the intense stress of space flight (Payne et al., 1999; Pierson et al., 2005) and Antarctic expeditions (Mehta et al., 2000a) result in higher EBV antibody titers and decreased EBV-specific T-cells responses. Elevated EBV antibody titers have also been reported in the context of stress associated with rapid economic and cultural changes in communities in Siberia and Samoa (McDade et al., 2000; Sorensen et al., 2009). In addition to effects of objective stressors, impaired control of latent viruses has been associated with psychological characteristics including anxiety, tendency toward emotional repression, and loneliness (Glaser et al., 1985; Esterling et al., 1990, 1993). Conversely, greater vigor and social support have been associated with lower EBV antibody titers in the context of major life stressors (Lutgendorf et al., 2001; McDade, 2001; Fagundes et al., 2011).

Although chronic stress may contribute to racial disparities in diverse health outcomes (Shonkoff et al., 2009), studies of racial differences in EBV reactivation are limited. In a study of adults ages 25–90, greater viral reactivation was evidenced among Blacks compared to non-Hispanic Whites (Stowe et al., 2010). In addition, a study of women of childbearing age demonstrated that perceived racial discrimination was associated with greater EBV reactivation (Borders et al., 2010). Thus, though limited, available evidence suggests that race and racial discrimination affect EBV reactivation.

It has been suggested that EBV reactivation is greater during pregnancy than non-pregnancy due to pregnancy-related suppression of the cellular immune system (Purtilo and Sakamoto, 1982). However, to our knowledge, there are no available data comparing non-pregnant women to women across the course of pregnancy. These data would provide insight into typical versus atypical immune adaptation as pregnancy progresses.

Data on effects of psychosocial factors on EBV reactivation during pregnancy are also limited. One study of pregnant women in their first trimester reported greater EBV reactivation among those with a diagnosis of clinical depression versus a well-matched non-depressed comparison group (Haeri et al., 2011). Considering the large literature in non-pregnant adults showing effects of psychosocial stress on EBV reactivation and unique immune environment in pregnancy, greater explication of stress-immune relationships in pregnancy is warranted.

As described, EBV reactivation can provide a marker of suppressed cell-mediated immunity. As such, elevated EBV VCA IgG antibody titers may serve as a non-causal marker of increased risk of adverse birth outcomes. Moreover, reactivation of latent herpesviruses has been associated with inflammation (Bennett et al., 2012). This represents one potential causal pathway by which EBV reactivation may affect birth outcomes.

Building upon the limited existing literature, this study examined effects of gestational age, race, and subjective stress on EBV virus capsid antigen immunoglobulin G (VCA IgG) antibody titers longitudinally across pregnancy and postpartum. Considering expected down-regulation of cell-mediated immune function during pregnancy, we hypothesized that EBV VCA IgG antibody titers would increase from the 1st to 3rd trimesters and be lower postpartum than during pregnancy. Reflecting the chronic stress of racial minority status, we hypothesized that African-American women would exhibit elevated EBV VCA IgG antibody titers compared to Whites, and that subjective stress, particularly perceived racial discrimination, would exacerbate this effect. Finally, we examined the association of EBV VCA IgG antibody titers with serum proinflammatory cytokines to test the hypothesis that EBV reactivation may promote inflammatory activity.

2. Methods

2.1. Study design

Sixty pregnant women were recruited from the Ohio State University Medical Center (OSUMC) Prenatal Clinic. Study visits were conducted during the 1st, 2nd, and 3rd trimesters and at 4–9 weeks postpartum. At each visit, women provided a blood sample and completed measures of psychosocial stress and health behaviors. Women were excluded from analyses if they missed more than one of the three prenatal study visits ($n = 3$). One woman (who was White) was excluded from analyses due to EBV seronegative status at all four study timepoints, resulting in a final sample of 56 women.

2.2. Participants

All women were born and raised in the United States. Women were not eligible if they had current hypertension, diabetes, chronic conditions with implications for immune function (e.g., rheumatoid arthritis, multiple sclerosis, or human immunodeficiency virus), fetal anomaly, illicit drug use or more than two alcoholic drinks per week during pregnancy (per self-report or medical record). Women reporting acute illness (e.g., cold or flu-like symptoms) or antibiotic use within 10 days of a study visit were rescheduled. Each completed informed consent and received modest compensation. The study was approved by the OSU Biomedical Institutional Review Board.

2.3. Demographics and birth outcomes

Age, race/ethnicity, marital status, education, annual family income, gravidity, and parity were collected by self-report. Pre-pregnancy body mass index (BMI; kg/m^2) was calculated using

self-reported pre-pregnancy weight and height measured at the first visit. Gestational age at delivery was determined by medical record review.

2.4. Psychosocial measures

Questionnaires were administered per the schedule shown in Table 1. The Experiences of Discrimination (EOD) scale is a 9-item measure assessing the occurrence and frequency of discrimination due to race/ethnicity. Specifically, participants indicated whether they have experienced discrimination over their lifetime (Yes or No) in the following settings: (1) at school, (2) getting hired or getting a job, (3) at work, (4) getting housing, (5) getting medical care, (6) getting service in a store or restaurant, (7) getting credit, bank loans or a mortgage, (8) on the street or in a public setting, (9) from the police or in the courts. For items endorsed, participants rate the frequency of this occurrence: once, 2–3 times, or 4+ times. This scale has high test–retest reliability and predictive validity for health outcomes in Black adults (Krieger, 1990; Krieger and Sidney, 1996; Krieger et al., 2005). Moreover, validation studies indicate that scores are not related to social desirability (Krieger et al., 2005).

The Center for Epidemiological Studies Depression Scale (CES-D) is a 20-item measure of cognitive, emotional, and somatic symptoms of depression (Radloff, 1977). The CES-D is predictive of pre-term birth and immune parameters in pregnant women (Orr et al., 2002; Christian et al., 2009, 2010; Li et al., 2009; Phillips et al., 2010). The 10-item Perceived Stress Scale (PSS), is a well-validated measure which assesses a construct independent of depressive symptomatology (Cohen et al., 1983). Scores have been associated with maternal neuroendocrine function (Wadhwa et al., 1996; Hobel et al., 1999). The 6-item short form of the State-Trait Anxiety Inventory (STAI) was used to assess state anxiety (Spielberger, 1989; Marteau and Bekker, 1992). The STAI shows strong criterion, discriminant, and predictive validity in perinatal populations (Meades and Ayers, 2011). The Revised Prenatal Distress Questionnaire (NUPDQ) is a 17-item measure of pregnancy-specific stress including physical discomforts, financial resources to care for children, and pain during delivery (Lobel, 1996).

2.5. Health behaviors

The Pittsburgh Sleep Quality Index (PSQI), was administered at each visit (Buysse et al., 1989). A score > 5 is indicative of clinically disturbed sleep. Smoking, exercise and prenatal vitamin use were assessed at the first study visit. Women were classified as current, past, or never smokers. Exercise was operationalized as the frequency of engaging in vigorous physical activity long enough to build up a sweat. Prenatal vitamin use was defined as never, 1–3 days per week, 4–6 days, and 7 days.

2.6. EBV reactivation

Serum was assayed for EBV virus capsid antigen (VCA) IgG antibody titers using Euroimmun EBV ELISA plates (Morris Plains, NJ).

Table 1
Psychosocial questionnaires.

Measure	Trimester			Postpartum
	1st	2nd	3rd	
Experiences of Discrimination (EOD)	X			
State Anxiety Inventory (STAI)	X	X	X	X
Center for Epidemiologic Studies Depression Scale (CES-D)	X	X	X	X
Perceived Stress Scale (PSS)	X	X	X	X
Pittsburgh Sleep Quality Index (PSQI)	X	X	X	X
Revised Prenatal Distress Questionnaire (NUPDQ)	X	X	X	

EBV VCA IgG antibody titers were assessed following company instructions with some modifications (Fagundes et al., 2011). Specifically, for each ELISA plate, three controls that were included in each kit (one positive sample, one negative sample, and three calibrators) were run in duplicate. Samples were initially diluted 1:101 with a dilution buffer according to the recommended protocol provided by the company. Six serial twofold dilutions of each sample were assayed. The last dilution factor with a positive IgG value determined the IgG antibody titer. Calculated viral titers for each sample were plotted and samples were rerun if the end point did not fall within the linear range ($\pm 15\%$).

2.7. Proinflammatory markers

Serum levels of interleukin (IL)-6, tumor necrosis factor (TNF)- α , IL-8, and IL-1 β were assayed in duplicate with ultra-sensitive multiplex kits from Meso Scale Discovery (MSD) and chemiluminescence methodology using the Immulite 1000 (Siemens Healthcare Diagnostics, Inc., 1717 Deerfield Rd., Deerfield, IL). Limits of detection were 0.61 pg/ml for IL-6, 2.4 pg/ml for TNF- α , 0.3 pg/ml for IL-8, and 0.61 pg/ml for IL-1 β . All values were above the limits of detection. Assays were conducted after all data collection was complete; all assays were batched by subject and all kits were from the same lot.

2.8. Statistical analyses

We used linear mixed models to analyze EBV VCA IgG antibody titers. The linear mixed models account for correlation in measures from the same woman over time by including a random subject effect. These models are capable of incorporating subjects with missing data points because of the restricted maximum likelihood estimation method used to fit the models. EBV titer values were log-transformed (base 10) to better satisfy normality assumptions.

We first evaluated changes in EBV VCA IgG antibody titers across pregnancy by including visit as a categorical independent variable. Differences between visits were compared by contrasts of mixed model parameters for each pair of visit timepoints, using the Tukey adjustment for multiple comparisons. Next we examined racial differences in EBV VCA IgG antibody titers by adding race (African-American or White) to the model along with the interaction between race and visit. Differences between races were tested at each visit by contrasts within the mixed model. The African-American women were then subdivided by a median split on scores on the Experiences of Discrimination (EOD) measure and EBV VCA IgG antibody titers for the resulting groups were compared at each visit within the mixed model. In secondary analyses, we used generalized linear models with a logit (dichotomous outcomes) or cumulative logit (ordered categorical outcomes) link to compare health behaviors by race at each timepoint.

Linear mixed model analyses were conducted to examine the relationship between EBV VCA IgG antibody titers and serum inflammatory markers. Correlations at each individual visit were tested as contrasts within the model. Serum inflammatory marker data were log-transformed. No outliers (datapoints ± 3 SD from the mean) were present; therefore all available data were included. All analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, 2009).

3. Results

3.1. Sample characteristics

Women completed study visits in the 1st trimester (Mean = 11.4 weeks gestation, SD = 2.2), 2nd trimester (Mean = 23.4 weeks

gestation, SD = 2.3), 3rd trimester (Mean = 31.9 weeks gestation, SD = 1.8), and postpartum (Mean = 5.3 weeks, SD = 1.3). Complete data at all four study timepoints were available for 64% ($n = 36$) of women. Data were missing at one study timepoint for 27% ($n = 15$) and at two study timepoints for 9% ($n = 5$). In this sample, 11.1% (2/18) of White women and 10.5% (4/38) of African-Americans delivered preterm (<37 weeks gestation). Two preterm deliveries were medically-indicated due to preeclampsia (1 African-American, 1 Hispanic White).

3.2. Demographics and health behaviors

In this sample, 64% ($n = 36$) were African-American, 32% ($n = 18$) were White, including two Hispanics, and 4% ($n = 2$) reported both African-American and White race. For analytic purposes, these two women were categorized as African-American, as their experiences of racial discrimination were expected to better approximate those of African-Americans versus Whites (Campbell and Herman, 2010). Whites were more likely to be married than African-Americans ($p = .02$). African-Americans and Whites did not differ in other demographic characteristics (Table 2) or assessed health behaviors (Table 3).

3.3. Reported racial discrimination

Among the African-American women, 21 reported no experience of racial discrimination in the major life domains assessed; 17 reported experiencing discrimination in one or more life domains. Women were divided into two groups: those who reported racial discrimination (high discrimination) and those reporting no occurrence (low discrimination). Among those in the high discrimination category, four reported discrimination in one life domain, eight reported discrimination in two life domains, and five reported discrimination in three or more life domains. The domains in which discrimination was reported, in order of frequency of endorsement were: getting service in a store or restaurant ($n = 9$), on the street or in a public setting ($n = 9$), at school ($n = 5$), at work ($n = 5$), getting hired or getting a job ($n = 4$), getting housing ($n = 4$), getting credit, bank loans, or a mortgage ($n = 2$), from the police or in courts ($n = 2$), and getting medical care ($n = 1$).

3.4. Stage of gestation and EBV reactivation

We utilized linear mixed models to assess changes in EBV VCA IgG antibody titers longitudinally across pregnancy and postpartum, permitting use of all available data. Contrary to prediction, EBV VCA IgG antibody titers were significantly lower in the 3rd trimester as compared to the 1st trimester [\log_{10} model-adjusted mean titer (95% CI): 2.94 (2.83, 3.05) vs. 3.01 (2.90, 3.12), adjusted $p = .002$]. Postpartum EBV VCA IgG antibody titers [2.98 (2.87, 3.10)] approached the peak level observed in the 1st trimester, but were not significantly different than any of the pregnancy timepoints (adjusted $p = .10$ for 3rd trimester vs. postpartum).

3.5. Racial difference in EBV reactivation

Shown in Fig. 1, linear mixed models demonstrated that EBV VCA IgG antibody titers were significantly greater among African-American versus White women during each trimester of pregnancy as well as postpartum [\log_{10} model-adjusted mean titers (95% CIs), African-American vs. White: 1st trimester 3.13 (3.02, 3.26) vs. 2.73 (2.56, 2.90); 2nd trimester 3.13 (3.01, 3.25) vs. 2.62 (2.45, 2.80); 3rd trimester 3.09 (2.97, 3.20) vs. 2.64 (2.47, 2.81); postpartum 3.14 (3.02, 3.26) vs. 2.66 (2.48, 2.83), $ps < .001$].

3.6. Psychosocial stress and EBV reactivation

EBV VCA IgG antibody titers did not differ based on depressive symptoms, perceived stress, state anxiety, or pregnancy-specific stress during pregnancy or postpartum in the full sample ($ps > .11$) or among African-Americans when assessed separately ($ps > .17$). African-American women were classified as high versus low based on median split in terms of the number of situations in which they reported discrimination on the EOD scale. In a mixed linear model, those reporting high versus low discrimination showed significantly higher EBV VCA IgG antibody titers during the first ($p = 0.03$) and second trimesters of pregnancy ($p = .04$; Fig. 1). A similar, though non-significant, pattern was observed in the 3rd trimester ($p = .12$) and at postpartum ($p = .06$). When compared to White women, both groups of African-American women had elevated EBV VCA IgG antibody titers at all three trimesters

Table 2
Demographic characteristics.

	Total ($n = 56$)	African-American ($n = 38$)	White ($n = 18$)	African-American vs. White
Age [Mean (SD)]	23.7 (3.76)	23.61 (3.5)	23.89 (4.35)	$t(54) = 0.12, p = .80$
Marital Status [n (%)]				$\chi^2(1) = 5.21^a, p = .02^*$
Married	7 (12.5%)	2 (5.3%)	5 (27.8%)	
In a relationship	35 (62.5%)	26 (68.4%)	9 (50.0%)	
Single	14 (25.0%)	10 (26.3%)	4 (22.2%)	
Education [n (%)]				$\chi^2(1) = 0.17^b, p = .68$
Less than high school	16 (28.6%)	11 (28.9%)	5 (27.8%)	
High school graduate	16 (28.6%)	10 (26.3%)	6 (33.3%)	
Some college	18 (32.1%)	14 (36.8%)	4 (22.2%)	
College degree (2 or 4 y)	6 (10.7%)	3 (7.9%)	3 (16.7%)	
Income [n (%)]				$\chi^2(1) = 0.29^c, p = .60$
<\$15,000	37 (66.1%)	26 (68.4%)	11 (61.1%)	
\$15,000–29,999	13 (23.2%)	11 (28.9%)	2 (11.1%)	
≥\$30,000	6 (10.7%)	1 (2.6%)	5 (27.8%)	
Primigravid [n (%)]	4 (7.1%)	2 (5.3%)	2 (11.1%)	$p = .587^d$
Nulliparous [n (%)]	6 (10.7%)	4 (10.5%)	2 (11.1%)	$p = 1.0^d$
BMI [Mean (SD)]	29.51 (8.21)	30.01 (8.13)	28.45 (8.51)	$t(54) = -0.66, p = .51$

* $p < .05$.

^a Married versus unmarried.

^b High school versus greater education.

^c Above versus below \$15,000.

^d Fisher's exact test.

Table 3
Health behaviors.

	Total (n = 56)	African-American (n = 38)	White (n = 18)	African-American vs. White
<i>Smoking status</i>				$\chi^2(1) = .24^a, p = .71$
Current	10 (17.9%)	6 (15.8%)	4 (22.2%)	
Past	16 (28.6%)	12 (31.6%)	4 (22.2%)	
Never	30 (53.6%)	20 (52.6%)	10 (55.6%)	
<i>Impaired sleep quality^b</i>				Generalized linear model
1st Trimester	.57	.57	.58	$p = .98$
2nd Trimester	.61	.58	.67	$p = .53$
3rd Trimester	.66	.63	.72	$p = .50$
Postpartum	.71	.71	.72	$p = .93$
<i>Prenatal vitamin use</i>				$\chi^2(1) = 2.95^c, p = .08$
Never	23 (41.1%)	18 (47.4%)	5 (27.8%)	
Some days (1–3/week)	5 (8.9%)	4 (10.5%)	1 (5.6%)	
Most days (4–6/week)	7 (12.5%)	5 (13.2%)	2 (10.5%)	
Every day (7 days/week)	21 (37.5%)	11 (28.9%)	10 (55.6%)	
<i>Exercise</i>				$\chi^2(1) = 2.29^d, p = .13$
Less than once per month	18 (32.1%)	14 (36.8%)	4 (22.2%)	
Once per month	8 (14.3%)	4 (10.5%)	4 (22.2%)	
2–3 Times per month	7 (12.5%)	7 (18.4%)	0 (0%)	
Once per week	13 (23.2%)	8 (21.1%)	5 (27.8%)	
More than once per week	10 (17.9%)	5 (13.2%)	5 (27.8%)	

^a Current smoker versus current non-smoker.
^b Pittsburgh Sleep Quality Index (PSQI) Score > 5, model estimated proportion.
^c Never/some days versus most days/every day.
^d Greater or equal to once per week versus less exercise.

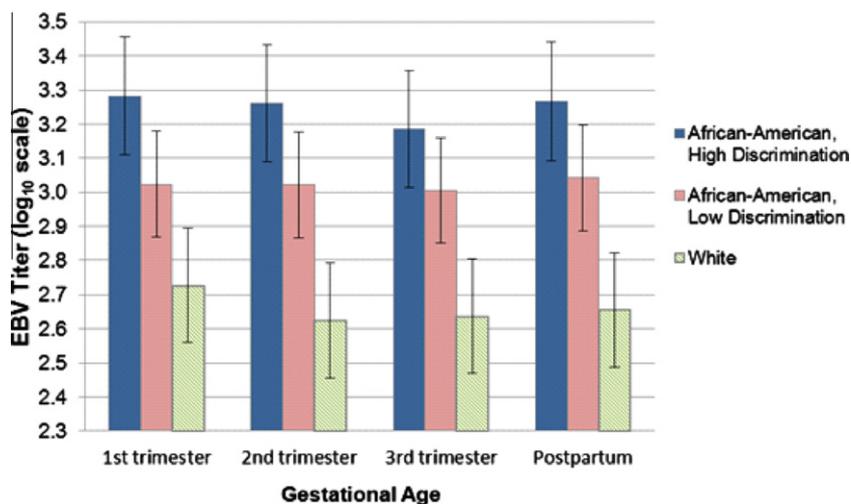


Fig. 1. Epstein-Barr virus (EBV) VCA IgG antibody titers during pregnancy and postpartum (Means and 95% confidence intervals.) In the sample overall, EBV VCA IgG antibody titers were lower during the 3rd trimester as compared to the 1st trimester ($p = .002$). Compared to White women ($n = 18$) African-American women ($n = 38$) exhibited significantly higher EBV VCA IgG antibody titers at each stage of gestation and at postpartum ($ps < 0.001$). This effect was most pronounced among African-American women endorsing high racial discrimination ($n = 17$) versus those endorsing low racial discrimination ($n = 21$) [$p = .03$ (1st), $.04$ (2nd), $.12$ (3rd), $.06$ (postpartum)].

and at postpartum [high discrimination: $ps < .001$; low discrimination: $p = .01$ (1st), $.001$ (2nd), $.002$ (3rd), $.001$ (postpartum); Fig. 1].

3.7. EBV VCA IgG antibody titers and serum inflammatory markers

To determine the extent to which EBV reactivation may promote inflammatory activity, we examined EBV VCA IgG antibody titer status in relation to serum IL-6, IL-8, TNF- α , and IL-1 β . Results of linear mixed model analyses indicated that none of the serum inflammatory cytokines were significantly associated with EBV VCA IgG antibody titer status at any of the visits ($ps > 0.18$).

3.8. Race, stress, and health behaviors

As described above, African-American women did not differ significantly from Whites on any health behaviors assessed

(Table 3). Among African-American women, those reporting high versus low discrimination also did not differ in health behaviors: current smoking ($\chi^2(1) = .08, p = .78$), vigorous exercise (once per week versus less; $\chi^2(1) = .02, p = .90$), prenatal vitamin use ($\chi^2(1) = 1.45, p = .22$), or rates of clinically disturbed sleep during any trimester of pregnancy (PSQI score > 5; $ps > .17$).

4. Discussion

This study provides novel data on longitudinal stability in EBV VCA IgG antibody titers across the course of pregnancy. Based on the limited prior literature (e.g., Purtilo and Sakamoto, 1982), we hypothesized that EBV antibody titers would be greater with increasing gestational age due to suppression of cell-mediated immune function. Contrary to prediction, EBV VCA IgG antibody titers were significantly lower in the 3rd trimester of pregnancy as

compared to the 1st trimester. Overall, the stability of EBV VCA IgG antibody titers across pregnancy was remarkable. Across the course of the study, including postpartum follow-up, no woman exhibited a change of more than one dilution in EBV VCA IgG antibody titers.

As an opportunistic latent virus, factors inducing immunosuppression allow EBV to reactivate. Reactivation can be associated with the release of viral antigens and complete infectious virus particles, resulting in the initiation of an antibody response. Thus, EBV VCA IgG antibody titers can provide an indirect measure of cell-mediated immune function. Reactivation may also result in the expression of viral proteins which by themselves may induce immune dysregulation (Glaser et al., 2006). In non-pregnant adults, increases in EBV VCA IgG antibody titers are commonly interpreted as an indication of impaired cell-mediated immunity (e.g., Glaser and Kiecolt-Glaser, 1994; Stowe et al., 2001) and decreases in antibody titers as indicative of improved cell-mediated immunity (e.g., Esterling et al., 1992).

In the context of pregnancy, the observed decrease in antibody titers in later pregnancy may be due to one or more factors. Prior research has demonstrated the overall serum concentrations of IgG, as well as IgA and IgM, decrease during normal pregnancy (Amino et al., 1978; Malek et al., 1996). It has been suggested that this may result from suppression of humoral immunity (Amino et al., 1978; Malek et al., 1996). In addition, IgG is actively transported across the placenta, with the largest transfer occurring in the third trimester (Simister 2003; Kane and Acquah, 2009). Although blood volume increases by 40–50% during pregnancy (Monga, 2009), prior data suggest that hemodilution only modestly contributes to the overall decrease in IgG observed in pregnancy (Amino et al., 1978). Moreover, in the current dataset, decreased EBV VCA IgG antibody titers were observed only in the 3rd trimester. If this effect were due to hemodilution, decreases in the 2nd trimester would be expected as well, as blood volume increases considerably through approximately week 28 after which levels remain relatively stable.

Therefore, while scientifically accurate, the finding of decreased EBV VCA IgG antibody titers in late pregnancy is unexpected and not easily explained considering the complex and multifactorial physiological changes that occur during pregnancy. Given the unique context of pregnancy, this study would have been strengthened by the inclusion of direct measures of viral replication. Although EBV antibody titers provide a useful indirect measure of reactivation, it is not possible to determine whether the observed decrease in antibody levels in late pregnancy was due to a general inhibition of antibody production or a change in the expression of latent virus. A more direct method of determining viral reactivation/replication is via measurement of viral DNA levels. This should be considered in future studies.

In terms of racial differences, as hypothesized, African-Americans exhibited substantially higher EBV VCA IgG antibody titers than Whites during each trimester of pregnancy and postpartum. Supporting a role for chronic stress in this relationship, this effect was significantly stronger among African-American women reporting greater lifetime occurrence of racial discrimination.

Most prior studies of EBV reactivation in non-pregnant adults have not included a sufficient number of African-Americans to examine racial differences. As reviewed, one study reported greater viral reactivation among Black adults ages 25–90 as compared to non-Hispanic Whites (Stowe et al., 2010). Also, among non-pregnant women, perceived racial discrimination was associated with greater EBV reactivation (Borders et al., 2010). A study of depressed versus non-depressed pregnant women found no difference in EBV reactivation by race (Haeri et al., 2011). However, only 9.5% of women were African-American and only 14% were very low

income (i.e., Medicaid recipients). The current sample was 68% African-American and 66% reported an annual family income <\$15,000. Thus, the power to detect effect of race and racial discrimination was likely enhanced in the current study by the high representation of African-Americans as well as greater vulnerability to stressors among economically disadvantaged women.

The current dataset did not provide an adequate sample size to examine EBV antibody titers in relation to adverse birth outcomes, such as preterm birth; only six preterm births occurred in this sample. Data relating EBV reactivation to adverse pregnancy outcomes is limited mostly to case reports of primary infection (Brown and Stenchever, 1978; Goldberg et al., 1981; Fleisher and Bolognese, 1983, 1984; Schuster et al., 1993). While primary infection occurs in only 0.05–1.5% of pregnancies (Le et al., 1983; Eskild et al., 2005), >90% of women carry EBV in a latent state and are therefore susceptible to reactivation. At least one study found no association between EBV reactivation and length of gestation or congenital abnormalities (Avgil et al., 2008). Two studies have linked EBV reactivation to adverse pregnancy outcomes including stillbirth, birth defects, shorter gestation, and lower birth weight (Icart et al., 1981; Eskild et al., 2005). Also, some data link maternal EBV reactivation to risk of leukemia and testicular cancer in offspring (Lehtinen et al., 2003; Tedeschi et al., 2007; Holl et al., 2008). Future studies with considerably larger sample sizes are needed to examine the current racial differences in EBV antibody titers in relation to birth outcomes.

Available data do not indicate whether EBV may play a causal role in adverse perinatal health outcomes or if it serves as a general marker of a pathological process (i.e., impaired cellular immune competence). Work from our laboratory has shown that at least one EBV encoded early protein, dUTPase, can induce the synthesis of several proinflammatory cytokines including IL-8, IL-6 and IL-1 β (Glaser et al., 2006). The target cells are dendritic cells and macrophages (Ariza et al., 2009). As reviewed, a growing literature supports a role for inflammation in the relationship between stress and preterm birth (Coussons-Read et al., 2005, 2007; Ruiz et al., 2007; Paul et al., 2008; Christian et al., 2010; Blackmore et al., 2011; Cassidy-Bushrow et al., 2012; Coussons-Read et al., 2012). There were no significant associations between EBV VCA IgG antibody titers and serum proinflammatory cytokines in the current sample, suggesting that EBV reactivation did not significantly promote inflammatory activity. However, further work in larger samples is needed to understand these interactions and implications for pregnancy.

We examined health behaviors as a potential mediator of the relationship between stress and EBV VCA IgG antibody titers. Women did not differ in body mass index, smoking, exercise, sleep, or prenatal vitamin use based on either race or perceived racial discrimination. Thus, health behaviors did not explain the observed differences in EBV VCA IgG antibody titers, supporting the role for direct physiological pathways in the relationship between stress and immune dysregulation. A limitation of these data is that they were obtained by self-report. All self-reported pre-pregnancy weights were plausible given the woman's weight as measured by scale at the first study visit. However, women are more likely to under-report rather than over-report their pre-pregnancy weight (Kovalchik, 2009). Similarly, self-reported smoking among pregnant women likely underestimates true smoking behavior (Shipton et al., 2009).

Contrary to prior studies, aside from racial discrimination, other stress parameters (e.g., depressive symptoms, perceived stress) were not associated with EBV VCA IgG antibody titers in this sample. This may reflect statistical power; these effects may be smaller than those exerted by race and racial discrimination, thereby requiring a larger sample size to permit detection. In addition,

greater range and representation of women with lower stress may provide greater power to detect effects of other stress parameters.

Our goal in utilizing EBV VCA IgG antibody titers as a marker in the current investigation was to use this as an indicator of cellular immune competence. That is, we hypothesize that impaired cell-mediated immune function, rather than EBV reactivation *per se*, may contribute to adverse perinatal health outcomes. However, it is possible that EBV itself may play a causal role. Given this possibility, effects of stress and pregnancy status on other latent viruses warrant attention as well, including herpes simplex virus (HSV) I and II, varicella-zoster virus (VZV), and cytomegalovirus (CMV). For example, CMV and HSV-1 are carried latently by approximately 60% of adults in the US by age 40 (Staras et al., 2006; Xu et al., 2006) and both may be reactivated in conditions of stress (Glaser et al., 1985; Schaeffer et al., 1985; Mehta et al., 2000b). Moreover, inflammation associated with viral reactivation may only be detectable in the context of reactivation of multiple herpesviruses (Bennett et al., 2012). Thus, assessment of seropositive status and reactivation of multiple herpesviruses would be informative in future studies.

The literature linking stress and immune parameters in human pregnancy is growing (Christian, 2012). Our data indicate that EBV VCA IgG antibody titers are significantly lower in later pregnancy as compared to early pregnancy. Compared to Whites, African-American women showed greater EBV VCA IgG antibody titers across pregnancy and postpartum. This difference was the most considerable among African-American women reporting greater racial discrimination, supporting a role for chronic stress in this association. As a possible indicator of stress-induced immune dysregulation during pregnancy, EBV provides a novel viral model which may elucidate biological pathways underlying racial disparities in perinatal health. Continued research is needed to determine the reliability of these findings in larger cohorts and the extent to which EBV reactivation may causally contribute to adverse pregnancy outcomes or instead serve as a marker of general immune dysregulation. In sum, this study provides novel data regarding the association of stage of pregnancy, race, and psychosocial stress with EBV VCA IgG antibody titers. These findings add to a growing literature showing that stress is associated with altered immune function during pregnancy.

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