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# Immune Changes and Dysphoric Moods across the Postpartum

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## Abstract

**Problem—**Little is known about postpartum immune recovery and relationships of common dysphoric moods, stress, immunology and endocrinology.

**Method of Study—**Healthy women (n=72) were followed for six postpartum months with immune and hormone measures and dysphoric moods and stress scales. A panel of cytokines produced in mitogen-stimulated whole blood assays were measured at each time, along with plasma levels of hsC-reactive protein (hsCRP), Interleukin-6 (IL-6), and a panel of hormones.

**Results—**Cellular immunity, measured by production of Interferon-gamma (IFNγ) and (Interleukin-2 (IL-2) from stimulated whole blood culture, was low in the early postpartum with changes by 3 months. Tumor necrosis factor alpha (TNFα) showed a similar pattern. Plasma levels of C-reactive protein and Interleukin-6 (IL-6) showed higher levels in the early postpartum. Mood disturbance scores dropped across the postpartum with a change in slope at 3 months. No significant relationships were found between immune, endocrine, and psychosocial measures.

**Conclusions—**Return to normal cellular immune function may take 3 to 4 months in the postpartum. Some aspects of early immunology (hsCRP and IL-6) probably reflect the latter stage of pregnancy, the stress of birth and the inflammation associated with involution. Dysphoric moods are higher in the early postpartum but are not related to immune factors or hormones.

### Keywords

cytokines; CRP; immunity; moods; postpartum

## Introduction

Pregnancy is characterized by profound changes in maternal physiology, which include alterations in both the innate and adaptive immune systems. There is evidence that Th1 cytokines are decreased in pregnancy with resultant Th2 dominance [1, 2]. Along with cellular immune changes, pregnancy is associated with upregulated innate immune processes [3]. While uncontrolled inflammation is clearly dangerous to pregnancy [4], new findings support that controlled, physiological important inflammation is critical to successful pregnancy.

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When labor and delivery occur, the loss of the placenta, which has orchestrated much of the local, and to some degree peripheral, immune changes of pregnancy, leads to a new immunophysiological state. Marked endocrine changes occur at birth which have been associated with postpartum mood disorders[5]. The postpartum may reflect aspects of pregnancy-associated alterations for an unknown period of time. However aspects of postpartum physiology do not just reflect immune "recovery" from pregnancy. Involution, lactation, immune protection of fetal microchimeric cells and return to reproductive fitness are characteristics of this period. Return to a "normal" prepregnant immune state is not well characterized and may take as long as one year after the birth [6]. Natural Killer cell cytotoxicity, suppressed during pregnancy, remains suppressed for over 6 months in postpartum women [7]. T cells expressing the CXCR3 or CCR4 marker, CD4+CD25+ cells (T regs) and HLA-DR+ cells increase across the postpartum. [8].

The interactions of immunology and behavioral phenomena (moods and stress) are also important to understand, as the postpartum is a stressful time of adjustment for women and is also characterized by higher than normal levels of depression and fatigue [9]. These behavioral phenomena may interact with the immune system and vice-versa [10]. The research questions asked in this study were 1: What are the levels of cytokines across the postpartum in healthy women? and 2) What are the relationships among dysphoric mood, stress, hormones and immune proteins ?

## Materials and methods

This study was approved by the university and clinical site's Institutional Review Boards and all women gave informed consent. Healthy postpartum women (N=568) were recruited during pregnancy (between 15 and 25 weeks) and then a random sample (N=72) was selected for postpartum follow-up. These women were the healthy comparison group in a parent study of postpartum thyroiditis. Women were visited by a nurse seven times, at week one postpartum and monthly for 6 months after birth with home visits, blood draws, physical measurements and questionnaires. Participants received a graduated honorarium, which totaled $200. The demographic questionnaire inventoried medications, health, exercise, smoking, breastfeeding status, and work. The Perceived Stress Scale (PSS) evaluated perceptions of stress. Participants also completed the Profile of Mood States (POMS)[11], a 65-item scale evaluating moods experienced during the past week. The POMS is composed of a total mood disturbance (TMD) score and six subscales: tension, depression, anger, vigor, fatigue, and confusion. Since the parent study investigated a range of dysphoric moods, a specific postpartum depression scale was not used.

Fifteen mLs of blood were collected in heparinized tubes by venipuncture from the antecubital space at a.m. home visits. Blood was kept cool and brought to the lab within 2-3 hours. A whole blood sample was used in an *ex vivo* culture, measuring cytokine production in mitogen-stimulated cultures. Whole blood (1.2 mls) was suspended in 4.8 mls of RPMI-1640 media with 10 ug/ml gentamycin, 5.0 ug/ml of phytohemagglutinin (PHA) (Sigma-Aldrich) and 5.0 ug/ml of lipopolysaccharide (LPS (ICN Biomedicals)) and placed in wells in a 16 well plate. The plates were incubated at 37° C with 5% $CO_2$ for 48 hours. The contents were centrifuged at 400g, and the supernatants were frozen at −80° C until

analysis. Cytokines (IL-2, IL-12, IL-10, IFN-γ, TNF-α, and IL-4) in the supernatants were measured in bead-based multiplex assays (Millipore, Billerica, Ma) according to kit directions on a Luminex-200 machine. Because of dilution issues, IL-6 was analyzed by ELISA (eBioscience, San Diego, CA). Plasma samples were also extracted, aliquotted and frozen at −80° C for IL-6 using Millipore kits and the Luminex-200 and hs-CRP was measured by ELISA (DRG, Mountainside, NJ) according to kit directions. Hormones were measured by ELISAs at months 1, 3 and 6, and included cortisol (ALPCO, St. Louis), estradiol and progesterone (DRG, Springfield, N.J.) and prolactin (Calbiotech, Spring Valley, CA).

Frequencies and percentages were generated for the demographic variables. There was little missing data and few dropouts for the psychosocial scales (mean number available across months 1 through 6 was 69). The average number of available blood samples across measurement times for the plasma assays was 64 and for the whole blood assays was 46. Reasons for this lower number included inadequate amounts of blood for this assay and occasional lab errors when the incubation endpoint was on weekends. Mean plots with error bars over months postpartum were produced for the outcomes variables of TMD, whole blood supernatant cytokine levels, plasma hsCRP and IL-6. Piecewise linear mixed models were applied to estimate the change in slopes at different time periods of months postpartum. First, linear mixed models were used for modeling the correlations among the repeated measures of the outcomes. We used AR(autoregressive) (1) correlation structure for all of our models. Second, piecewise models were selected because the mean plots showed change points at different time periods. Once we selected a change point, we created an indicator variable which is 0 before the change point and 1 afterwards. Then an interaction of time and this indicator variable is used as an additional predictor to the fixed effect of the linear mixed model. Model selection was performed by choosing a smaller AIC value. All analyses were performed using SPSS 22 and SAS 9.2. $p<0.05$ was used as the criteria for statistical significance.

## Results

A sample of 72 entered the study and 65 completed all six months of data collection. The sample was predominantly Caucasian (46%), with Hispanics (29%) and African Americans (13%) representing diversity. The majority were married (72%), had incomes over $40,000 (58%) and had college education (51%). At one week, 81% were breastfeeding, which dropped to 38.5% at six months. At one week 4.8% of mothers were working either part or fulltime. At three months, the percentage increased to 45%, and at 6 months, 61% of the sample was working, most in full-time jobs.

Table 1 depicts the Piecewise Linear Mixed Model Analysis for the significant variables. Figure 1 depicts the total mood disturbance (TMD) scale scores of the POMS across the postpartum. The range of scores on this instrument was between −10 and 82. We first did a log transform of the TMD which brought its distribution to nearly normal distribution. From its mean plot, we selected the change point occurring at postpartum month three. This two-segment model showed a significant change point at postpartum month three ($p=0.04$). The slopes of TMD before and after 3 month postpartum are −0.052($p=0.013$) and 0.0218

(p=0.256). The Cronbach's alpha in this sample was .89 for the TMD. In general all dysphoric mood subscales scores were higher in the early postpartum and dropped over time. Throughout the postpartum between 6 and 12.5 % (depending on the month of the postpartum) of the women had scores of 16 or above on the depression subscale of the POMS indicating possible postpartum depression. All of these women were referred to their health care providers and followed up. With regard to PSS scores, the Cronbach's alpha for this sample was 0.839. There was little difference in the means across time and the range was between 4 and 34.

Th1 cytokines, IL-2 and IFN-γ, produced in *ex vivo* cultures, showed the same pattern of lower values in the early postpartum that gradually increased over the postpartum months (Figures 2a and 2b). For IFN-γ levels, we used the square root transformation to improve the normality of the data as it did better than the log transformation. There was a significant change point at 3 months postpartum (p=0.04) and the before and after slope estimates are: 13.28 (p=0.007) and −0.96 (p=0.781 ). Similarly, the square root of the IL-2 had a significant change point at 4 month postpartum (p=0.0126). The before and after slope estimates are: 4.48(p=0.011) and −4.11(p=0.095). TNF-α showed gradually rising levels in the postpartum (Figure 2c). The log transformed TNF alpha showed a marginally significant change point at 4 months postpartum (p=0.08) and the slopes before and after the change point are: 0.1232 (p=0.012) and −0.0241 (p=0.691). The levels of all other cytokines from these cultures were not different. Plasma levels of hsCRP, on the other hand, were very high in the week after birth, and decreased over the first 2 postpartum months to near normal levels (Figure 3a). The change point model had the smallest Aikaika Information Criteria (AIC) when the change point is at 2 months postpartum with p<0.0001. From the week after birth to 2 months, the slope of CRP is −0.8263 (p<0.0001). Between 2 months to 6 months, the slope of hsCRP is −0.0358 with a p value of 0.561.

Most plasma cytokine levels did not vary across time. The exception to this was plasma IL-6 which dropped gradually across the postpartum (Figure 3b). We tested change point models for log transformed plasma IL-6 with change point at 3 months or 5 months but they had larger AIC values than the AIC value from a linear model with no change point. The linear model with no change point showed a slope estimate of −0.092 with p=0.001. None of the immune biomarkers differed by breastfeeding status. Cortisol and progesterone were stable over time. Estradiol increased and prolactin dropped in relationship to decreased exclusive breastfeeding (Figure 4). No demographic factors were significantly related to mood, stress, cytokines, or hormones. Pearson correlations between Log 10 transformed cytokine levels, hs CRP, BMI, POMS scores, and PSS scores were examined for each measurement time. Spearman correlations were done on the relationships among working status and the biomarkers, dysphoric moods, and stress. BMI was highly correlated with hsCRP across the postpartum and at week one the correlation was .61, p<.001 for hsCRP. The levels were not related to mood, hormone levels or cytokines. Geometric means of TNF-α and BMI were correlated (r=.17, p<.02). Hours working was associated with higher fatigue subscale scores at month 3 (σ=.25, p<.05) and with POMS-D scores at month 4 (σ=.26, p<.03). Otherwise, moods and stress were unrelated to immune factors across time.

## Discussion

During pregnancy the Th1 axis and NK cytotoxicity are suppressed. This Th1 and NK suppression appears to be maintained during the early postpartum, perhaps in order to allow fetal microchimeric cells to establish themselves in niches in the mother's body without maternal immune attack. At the same time, aspects of innate immunity appear upregulated, perhaps to provide increased surveillance and protection when there is reduced Th1 and NK competency. Another possibility is that the inflammation is associated with involution and remodeling of the uterus.[12] The low synthesis of Th1 cytokines in whole blood cultures in the early postpartum suggests a general suppression of aspects of the immune system. This could be due to a slow return to normal immunity after the suppression of pregnancy. It is reported that rheumatoid arthritis, a T-cell mediated autoimmune disease, undergoes a remission during pregnancy, presumably due to a Th-1 suppression, but returns or flares in the postpartum [13]. This reactivation typically occurs by the third postpartum month [14], which corresponds to the rising levels of IFN-γ and IL-2 in the current study. Aspects of innate immunity seems to be up regulated in the early postpartum as evidenced by higher hsCRP and IL-6 levels by about three months. However TNF-α level was low in the early months of the postpartum. TNF-α is reported to be stable across pregnancy[15], so this rise reflects some adjustments or responses to the postpartum period. Dysphoric moods were higher early in the postpartum. Most mothers returned to work around three to four months, a time point at which most of the immune changes and moods measured in the current study "normalized". We found little evidence that mood, stress, hormones or immunity are tightly linked during this period. It is possible that the first three to four months postpartum could be considered vulnerable, both emotionally based on many studies of postpartum depression, and immunologically, based on our cellular immune and psychosocial data, and previously reported data on NK suppression[16, 17]. Certain risks for infections exist in the early postpartum such as mastitis, endometritis, urinary tract infections and wound infections. In a recent large study of postpartum infections, one or more infections were experienced by 10.3% of the women in the first 2 months postpartum, with prevalence for mastitis 4.7%, urinary tract infection 3.0%, endometritis 2.0% and wound infections 1.8%[18]. These common infections may be related to the immune characteristics of the early postpartum.

Limitations of the study include the fact that pregnancy data or data at the time of birth was not obtained so that early values that e measured may well reflect the physiological effects of labor and birth. Another limitation is that the numbers were small and outliers with high values, particularly for hsCRP, could account for values that were unexpectedly higher even later in the postpartum.

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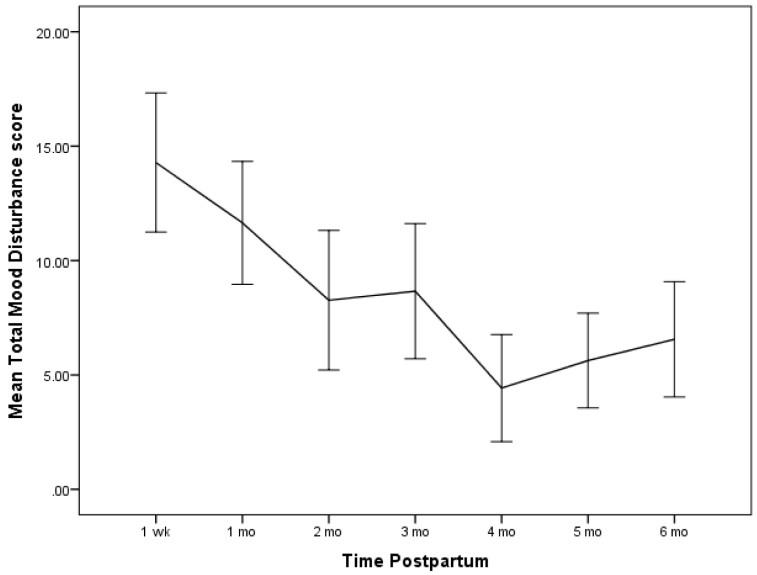
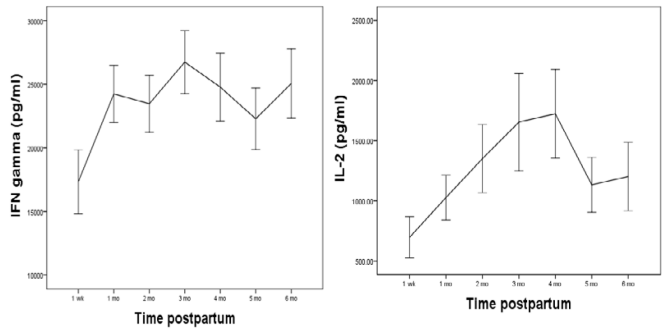
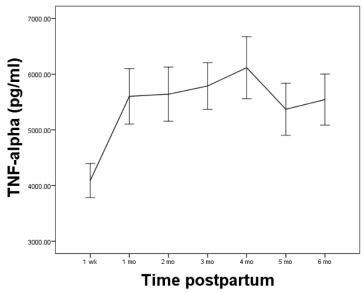



**Figure 1.**

Mean TMD by time; Error bars are standard errors if the means. Difference between week 1 and month 6 (t=1.97, df=124, p=.05).




2a 2b




2c

**Figure 2.**

Ex vivo whole blood culture synthesis of IFN-γ (2a),IL-2 (2b) and TNF-α (2c)over 6 postpartum months. Error bars are standard errors of the mean.

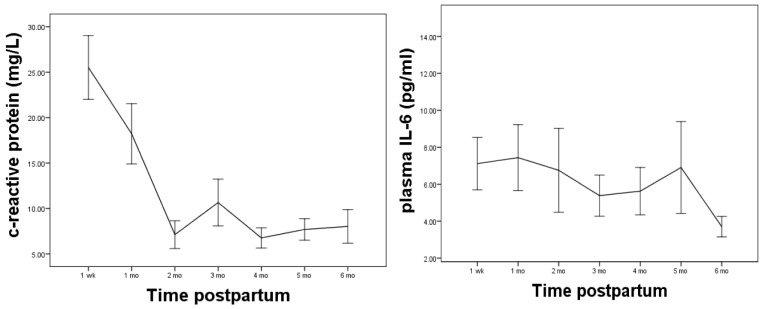



**Figure 3a and 3b.**
Mean Plasma CRP and IL-6 levels over 6 months postpartum.

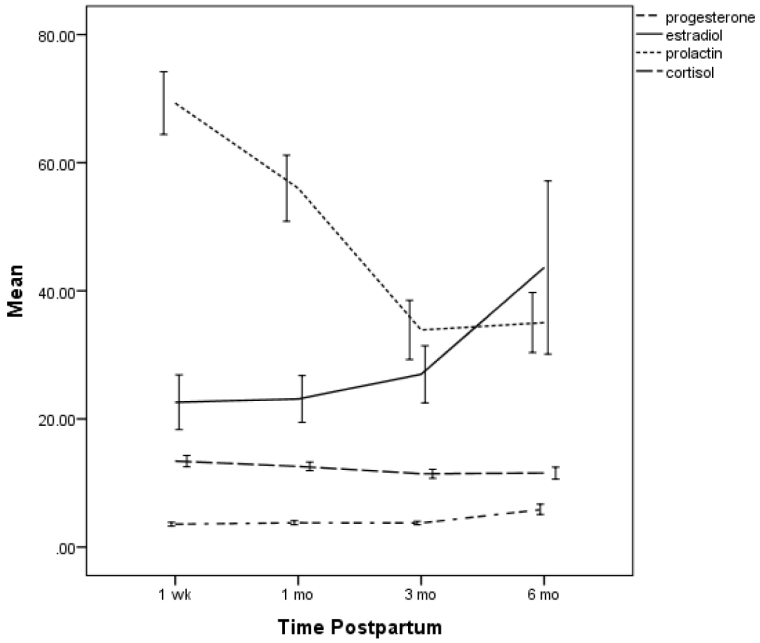



**Figure 4.**

Progesterone and Prolactin (ng/mL); Cortisol (μg/mL; Estradiol (pg/mL). All bars are standard errors of the mean.

## Table

for Piecewise Linear Mixed Model Analysis

| | Transformation | Change Point (p value) | Slope Before the Change Point (p value) | Slope After the Change Point (p value) |
|---|---|---|---|---|
| TMD | Log(.+40) | 4 months (p=0.04**) | −0.052(p=0.0132**) | 0.0218 (p=0.256) |
| IFN-γ | Square root | 3 months(p=0.04**) | 13.28 (p=0.0075***) | −0.96 (p=0.7815) |
| IL-2 | Log | 4 months (p=0.0126**) | 4.48(p=0.011**) | −4.11(p=0.095*). |
| TNF-α | Log | 4 months (p=0.08*) | 0.1232 (p=0.0122**) | −0.0241 (p=0.6913) |
| CRP | Log | 2 months (p<0.0001***) | −0.8263 (p<0.0001***) | −0.0358 (p=0.5614) |
| plasma IL-6 | Log | None | −0.092 (p=0.0009***) | |

* p<0.1

** p<0.05

*** p<0.01