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T-cell Defects and Postpartum Depression

Lauren M. Osborne, MD[1,2,*,+], **Janneke Gilden, MD**[3,+], **Astrid M Kamperman, PhD**[3], **Witte. J.G. Hoogendijk, MD, PhD**[3], **Julie Spicer, PhD**[5], **Hemmo A. Drexhage, MD, PhD**[4], **Veerle Bergink, MD, PhD**[3,5,6]

[1]Department of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA [2]Department of Gynecology & Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA [3]Department of Psychiatry, Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands [4]Department of Immunology, Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands [5]Department of Psychiatry, Icahn School of Medicine at Mt. Sinai [6]Department of Obstetrics & Gynecology, Icahn School of Medicine at Mt. Sinai

Abstract

Background.—Most studies of immune dysregulation in perinatal mood and anxiety disorders have focused on peripheral cytokines, but literature from non-perinatal mood disorders also implicates T-cell defects. We sought to characterize proportions of T-cell subtypes in women with postpartum depression.

Materials and Methods.—We enrolled 21 women with postpartum depression (PPD), 39 healthy postpartum controls, and 114 healthy non-postpartum women. Blood was collected in sodium-heparin EDTA tubes and was analyzed using flow cytometry. We conducted statistical tests including linear regression analysis that were aimed at determining differences in proportions of T cell populations among groups.

Results.—Mean counts of T-cells (all CD3+ T cells), T-helper cells, (CD3+CD4+ T cells), and T-cytotoxic cells (CD3+CD8+ T cells) were significantly increased in healthy postpartum women compared to healthy non-postpartum controls ($p < 0.001$, $p = 0.007$, and $p = 0.002$, respectively), but not in women with PPD. The increases in healthy postpartum women were driven by increases in T_H1 cells and T regulatory cells, increases that were nonexistent or attenuated in women with postpartum depression. Mean counts of CD4+ T-helper memory cells were also increased in healthy postpartum women ($p = 0.009$), but slightly decreased in women with PPD ($p = 0.066$), when compared to healthy non-postpartum controls.

[*]Corresponding author: Lauren M. Osborne, MD, Women's Mood Disorders Center, Johns Hopkins University School of Medicine, 550 N. Broadway, Suite 305, Baltimore, MD 21205, 410-955-9986 (phone), lmosborne@jhmi.edu.
[+]Co-first authors

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Supplemental information: Supplemental Figure 1; Supplemental Table 1; Supplemental Methods

Conclusions.—Our study confirms that the postpartum period in healthy women is a time of enhanced T cell activity. Women with postpartum depression failed to show physiological enhanced T-cell activity postpartum, and future research is needed to elucidate etiological mechanisms and consequences.

Keywords

T cells; immune; pregnancy; postpartum; depression; mood

INTRODUCTION

It is now well established that immune system dysregulation plays a role in major depressive disorder, with numerous studies linking peripheral cytokine alterations to depressed mood (Dowlati et al., 2010; Howren et al., 2009) and others showing that anti-inflammatory treatment can help some depressed patients (those with elevated inflammatory markers at baseline) (Haroon et al., 2018; Raison et al., 2013). Because immune dysregulation appears to play a role only for certain subsets of depressed people, it has been a logical progression to investigate the role of the immune system in psychiatric illness during the perinatal period. It is a time of known immune dysregulation and one of the few periods in life when there is an obvious biological trigger (parturition) that can be linked to psychiatric symptoms – specifically, to symptoms of perinatal depression, including the diagnosed depressive disorders that occur in up to 15–20% of women (Gaynes et al., 2005), with potentially devastating effects on women and families.

Early research on the physiological immune dysregulation of the peripartum focused on immune suppression during pregnancy, then on a supposed shift away from T-helper type 1 (T_H1) activity and toward T-helper type 2 (T_H2) activity (Larocca et al., 2008). More recent work has focused on a more complex model, with enhancement of innate immune barriers but reduced effectiveness of some elements of adaptive immunity across pregnancy (Chen et al., 2012; Holtan et al., 2015; Kraus et al., 2010; Pazos et al., 2012). In the postpartum, healthy women appear to have a rebound of adaptive immunity, in particular a rebound in T-cell activity, that has been identified in both animal and human literature (Bergink et al., 2013; Calcagni and Elenkov, 2006; Wegienka et al., 2011). Moreover, research on T-cell activity in depressive and anxiety disorders outside of pregnancy indicates deficiencies of T regulatory cells as well as dysregulation of T_H17 cells (Grosse et al., 2016; Osborne et al., 2019). T regulatory cells have also been shown to decrease in response to acute stress (Freier et al., 2010).

In light of this work on immune dysregulation in healthy pregnancy and in mood and anxiety disorders, numerous researchers have attempted to link immune dysfunction to both antenatal and postpartum depression, with mixed success. Most of these studies have focused on a small number of peripheral cytokines as markers of immune function (Osborne and Monk, 2013). A few recent studies have measured large numbers of peripheral markers and attempted to come up with summary variables (Brann et al., 2017; Edvinsson et al., 2017) – an improvement in technique that has nevertheless not yet yielded a useful measurement tool. In addition, many studies in the perinatal period have conflated antenatal

and postpartum depression, therefore making it difficult to draw conclusions about new-onset depression in the postpartum, a type of illness that may carry its own unique genetic signature representing distinct biological pathways (McEvoy et al., 2017).

Despite the relatively large number of studies – including our own (Osborne et al., 2018) – that have focused on peripheral cytokines, this may not be the ideal way to measure the relationship between immune function and psychopathology. It is unclear whether there is a correlation between levels of cytokines in the periphery and those in the central nervous system. One recent study, in perinatal depression, found no correlation between cytokines in the periphery and those measured in cerebrospinal fluid (Miller et al., 2019). Relatively few studies, by contrast, have examined either antenatal or postpartum depression in relationship to shifts among classes of immune cells. One early study found a negative association between T-cell count and dysphoria, but did not examine shifts among different types of cells within the T-cell compartment (Hucklebridge et al., 1994). Examining such shifts may give us important information about the biological mechanisms of perinatal depression, and may also yield novel therapeutic targets.

When first released from the thymus, T cells are “naïve”; upon presentation with antigen, they proliferate and differentiate into effector cells. Once the antigen has been cleared, 95% of the effector cells die, and the remainder take up long-term residence as memory cells (Mahnke et al., 2013). The effector subgroups are identifiable by the panel of cytokines they secrete. Cytotoxic T cells are characterized by the surface marker CD8+, and directly attack damaged cells. Helper T cells (CD4+) coordinate the immune response, and are further subdivided into several groups. T-helper 1 cells (T_H1) and T-helper 17 cells (T_H17) are involved in the activation of macrophages and secrete IFN-γ, among others, and IL-17, respectively. T-helper 2 cells (T_H2) cells secrete IL-4 and IL-5, among others, and are involved in the activation of B cells. The regulator subgroup is formed by the natural T-regulatory cells, which dampen the activity of T_H1, T_H2, and T_H17 cells (Mousset et al., 2019; Osborne et al., 2019; Piccinni, 2011; Saito et al., 2010). Our own group and one other have examined shifts among T cell classes in postpartum psychosis (PPP), another devastating but rare postpartum psychiatric illness (Bergink et al., 2013; Kumar et al., 2017). Our study showed that women with PPP failed to show the T-cell elevation characteristic of healthy postpartum women. Kumar’s group found that women with PPP failed to show an elevation in naïve T-helper cells that was characteristic of healthy postpartum women, but also showed higher levels of both cytotoxic T cells and T-regulatory cells. In addition, T-cell dysregulation has also been shown in numerous studies of mood disorders outside the perinatal period (Grosse et al., 2016; Snijders et al., 2019; Snijders et al., 2016).

Given this paucity of information, we therefore sought to expand the available evidence concerning immune cells and particularly T cell populations in postpartum depression by comparing women with severe PPD (with postpartum onset only) to both healthy postpartum controls and healthy women who were neither pregnant nor postpartum.

MATERIALS AND METHODS

Participants

This study protocol was approved by the institutional review board of the Erasmus Medical Center, Rotterdam (original protocol number MEC-2005226). After receiving a complete description of the study, all subjects provided written informed consent. Twenty-one (n=21) women with an acute postpartum onset of severe depression (PPD) were recruited from the Mother-Baby Inpatient Unit of the Department of Psychiatry of the Erasmus University Medical Center in Rotterdam, the Netherlands, between April 2007 and February 2012. All subjects were diagnosed according to DSMIV-TR (First, 1996) using the Structural Clinical Interview for DSM-IV (SCID – 1/P research version). Symptoms were additionally tracked using the Edinburgh Postnatal Depression Scale (EPDS). Those subjects diagnosed with PPD vi the SCID had a mean EPDS score of 18 (SEM 1.3). The relevant DSM-IV-TR diagnoses included both major depressive disorder alone (n=13) and major depressive disorder comorbid with anxiety disorders (n=8). Recent research in postpartum depression has indicated that there are distinct clinical phenotypes (Putnam et al., 2017), most of which include a significant anxiety component, and we therefore deemed it important to include both of these populations.

All women had an onset of symptoms within six months following delivery, and 14 had an onset within 4 weeks postpartum (67%). Those with a history of bipolar disorder, non-puerperal psychotic episodes, substance abuse, or psychiatric symptoms during pregnancy were excluded from the study. The median onset of symptoms occurred at day 7 postpartum (IQR 0.5–40.0). Mean time of blood collection occurred at day 61 postpartum. Of these 21 subjects, at the time of blood collection, nine were using benzodiazepines (median 2 days), two were using antipsychotics (seven and nine days), and one was using antidepressant medication (one day). Women admitted to our ward with depressive symptoms have an antidepressant-free observation period, which enabled us to enroll the majority of subjects before the start of antidepressant treatment. Eleven subjects had a previous history of non-puerperal depressive or anxiety symptoms. Physical examination and routine laboratory screening were performed at the time of study enrollment to confirm the absence of infection or other hematological abnormalities. All subjects were in an acute disease state at the moment of blood collection.

The healthy postpartum control group (HPC) consisted of 39 age-matched healthy postpartum women recruited between January 2009 and March 2012 (Erasmus MC, Rotterdam), with an EPDS score ≤10 (mean 3.8; SEM 0.4) at the time of postpartum blood sampling at mean 31 days postpartum.

One hundred twenty-four age-matched healthy non-postpartum women were included as an additional control group (HC). Inclusion criteria for both healthy postpartum and healthy non-postpartum women included the absence of any medical, neurologic, psychiatric, or autoimmune disorders, as well as having no current or recent clinical evidence of acute infection. All blood draws, from cases and controls, occurred in the morning, allowing us to minimize diurnal variations in immune factors.

Blood collection and preparation

Blood was collected in sodium-heparin tubes (30 ml) in the morning and transported to the laboratory at room temperature. Peripheral blood mononuclear cell (PBMC) suspensions were isolated using low-density gradient centrifugation by Ficoll (GE Healthcare, Uppsala, Sweden) within 8 hours. PBMCs were counted and frozen in medium (RPMI-1640 containing 25mM Hepes and UltraGlutamine (Lonza, Verviers, Belgium), with the addition of 10% fetal calf serum (Lonza), 10% dimethylsulfoxide (Merck, Hohenbrunn, Germany) and 1% Penicillin/Streptomycin) and stored in liquid nitrogen to enable testing case and control immune cells in the same experiment.

Flow cytometric analysis

PBMCs were defrosted and washed once with medium. Average recovery of cells after thawing was 82% and viability 97%, as determined by Trypan blue staining. Differences between different groups were not observed. Two different staining procedures were used: staining A determined percentages of different types of T cells, and staining B determined T helper subsets. (Details of the staining methods are included in supplemental information.)

All specific staining antibodies used are routinely tested for effectiveness by the manufacturer and titrated for optimal concentrations in our laboratory. Specificity of the staining antibodies was controlled using five isotype controls provided by the manufacturer (BD) and background positivity was negligible (between 0.2% and 1% of the specific staining depending on the isotype control, both in patient samples and controls).

Stained samples were analyzed by 8-color flow cytometry on a FACS Canto II (BD biosciences) and analyzed by FlowJo software (Tree Star, Ashland, OR, USA). Gating strategy for staining B is given in supplemental figure 1. T cell subsets of *staining B* were expressed as percentages of total lymphocytes, which could reliably be detected as a clear population in forward sideward scatter after the 4-hr culture.

Data exploration of flow cytometry data also revealed 10 outlier HC women (>3 SD). In accord with our statistician we decided to exclude all data of these healthy controls from further analysis including sociodemographic characteristics, leaving an N=114 in our HC group.

Statistical Analysis

Statistical analysis was performed using SPSS version 24.0. Sample characteristics were evaluated using Chi^2 tests or Fisher's exact test (if cell sizes were <5), and independent samples t-tests. Immune cell data were mean and standard error of the mean (SEM). To compare immune cell data between PPD women, HPC, women, and HC women, we used separate linear regression analyses (e.g. HC vs HPC and PPD; and HPC vs PPD). Comparisons with HC women were adjusted for body mass index (BMI). Comparisons between HPC and PPD women were adjusted for BMI, postpartum day of blood draw, and educational level. Confounders (e.g. BMI, postpartum day of blood draw, and educational level) were selected based on the existence of a significant associations with both predictor (sample) and outcome variable (immune cell data, see Supplemental Table 1). We report

Cohen's delta alongside p-values to represent the size of the difference. Normality of the data was explored visually using histograms and Q-Q plots, and tested statistically using Shapiro-Wilk tests. Normality of the error distribution was checked in the context of the regression analyses. Analyses were performed using untransformed immune cell data.

RESULTS

Sample Characteristics

We analyzed 21 PPD subjects, 39 HPC, and 114 HC. There were no differences in age, weight, ethnicity, marital status, gravidity, parity, delivery by Caesarean section, and delivery by vacuum extraction between women with PPD and HPC women (Table 1). Women with PPD had a higher BMI compared to HPC (p=.029). The HPC women were more likely to have education beyond high school (p=.020). Blood draw took place later after partus in PPD women than in HPC women (p<.001). The majority of HPC women were breastfeeding (71.8%), while very few PPD subjects were (4.8%, p<0.001). Demographic characteristics for HC women included only weight and BMI, hence we were unable to compare other demographic characteristics with the HC women.

Percentages of Overall T Cells among Peripheral Blood Mononuclear Cells (PBMCs)

Mean counts of T cells (all CD3+ T cells), T-helper cells, (CD3+CD4+ T cells) and T-cytotoxic cells (CD3+CD8+ T cells) were significantly increased in HPC compared to HC women ($p < 0.001$, $p = 0.007$, and $p = 0.002$, respectively; see Table 2). For PPD women, this was not the case; the mean count of CD8+ cells fell somewhat below that of HC women, while the mean count for CD4+ cells was intermediate between those of HC and HPC women, with no significant differences in either case. Mean counts of CD4+ T-helper memory cells (measured in staining B) were increased in HPC women compared to HC women, ($p = 0.009$), but somewhat decreased in the PPD women compared to HC women ($p = 0.066$). The mean count of T-helper naïve cells (calculated) was increased in PPD women as compared to HC ($p = 0.045$), with HPC women's levels in between the other two groups.

Percentages of T_H1, T_H2, T_H17, and T regulatory cells

We next sought to separate out T-cell subsets by testing in staining B for the capacity of CD4+ cells to produce the characteristic cytokines of T_H1, T_H2, and T_H17 cells, and for the intracellular presence of the transcription factor FOXP3 (characteristic of regulator cells). We saw substantial differences between HC and HPC and between PPD and HPC (Table 2). In HPC women, the rise in CD4+ T-helper cells was due to a rise in T_H1 cells and T regulatory cells ($p = 0.032$ and $p < 0.001$, respectively) compared to HC women. Mean counts of T_H2 and T_H17 cells did not differ between these two groups. In PPD women, by contrast, these rises were nonexistent or attenuated. T_H1 cells were even lower than in HC women ($p = 0.069$ vs. HPC and 0.031 vs. HC), and T regulatory cell counts were intermediate between the other two groups ($p = 0.044$ vs. HPC and 0.030 vs. HC). T_H2 cells again did not differ between groups, but T_H17 cells were somewhat lower in PPD women than in both other groups ($p = 0.365$ vs. HPC and 0.006 vs. HC). While not all differences reached statistical significance, effect sizes in some cases were substantial (see Table 2).

DISCUSSION

Our study clearly confirms that the postpartum period in healthy women is a time of altered immune activity, with increases in T cells compared to the non-postpartum period. The postpartum increases in T cells involved both the CD8+ cytotoxic and CD4+ helper T cells and were seen in both the T-helper naïve and memory populations. We also found that both the pro-inflammatory T_H1 and the immune suppressive T-regulatory cells were increased. Previous research, though scarce, has also shown that pregnancy and the postpartum condition persistently affect these lymphocyte populations. In the 1990s a Japanese group (Watanabe et al., 1997) showed that T-regulatory cells were increased in early pregnancy, while the number of T-cytotoxic cells decreased. In late pregnancy, T-helper cell numbers decreased. After delivery, T-helper cells, T-cytotoxic cells, and T-suppressor cells increased for a period of up to half a year. The investigators took these observations as indicating that early pregnancy alterations were related to the tolerance of the fetus, late pregnancy alterations to maintenance of pregnancy, and postpartum alterations to the combat of infections. The postpartum alterations could also explain the increased incidence of some autoimmune disorders postpartum (including multiple sclerosis and autoimmune thyroiditis) (Langer-Gould et al., 2010; Shi et al., 2009; Weetman, 2010).

Additional literature has supported a pattern of lymphocyte suppression during pregnancy followed by rebound after delivery in T-helper memory cells in particular. Matthiessen and colleagues found that T-helper memory cells decreased substantially during pregnancy and began to rebound early in the postpartum (at 2–7 days), still remaining lower than pre-pregnancy levels (Matthiesen et al., 1996). Kieffer and colleagues (Kieffer et al., 2017) looked much later in the postpartum (6 months) and found significantly *higher* proportions of T-helper memory cells in parous compared to nulligravid women, indicating that pregnancy persistently affects the pre-pregnancy CD4+ memory cell pool in human peripheral blood. Collectively, the two studies on T-helper memory cells support our own finding of a clear increase in T cells, including T-helper memory cells, in healthy women in the postpartum period, and it is tempting to speculate that these increases serve a physiological role in healing processes and in combatting infections in this vulnerable period and may also represent a tolerance induction toward paternal antigens (as speculated by (Kieffer et al., 2019)).

Women with postpartum depression, however, displayed a remarkably different pattern. The failure of postpartum depressed subjects to mount a physiological T-cell activation in the postpartum period is consistent with our earlier findings in postpartum psychosis subjects (Bergink et al., 2013). The abnormal apportioning of subsets here is also comparable to that found in our postpartum psychosis subjects: Cells with a T_H1 potential (IFN-γ production) were reduced in PPD compared to HPC and HC women controls, as were T_H17 cells, while cells with an immune suppressive capability (T regulatory cells) were significantly less activated as compared to the HPC women. It was particularly notable that T-helper memory cells failed to rise and were even reduced when compared to HPC women. Memory T cells, which remember previously encountered antigens through exposure to semen, fetal cells in pregnancy, or microchimerism, are thought to play a key role in fetal-maternal tolerance. Preeclampsia is considered to be a disease of immune maternal-fetal incompatibility, and a

recent study showed lower memory T cells not only during pregnancy but also postpartum in women who had preeclampsia during pregnancy compared to healthy controls (Kieffer et al., 2019). We earlier showed high co-occurrence of preeclampsia and postpartum mood disorders (Bergink et al., 2015), and lower memory T cells (and maternal-fetal incompatibility) might be evidence of a relationship in their underlying pathophysiology.

Some of these differences we found were more pronounced than others, and it may be that with a larger sample size these less pronounced differences would become more clear. The one category in which we saw not even a glimmer of difference between the two postpartum groups was in the T_H2 cells, indicating that this is primarily a story of cells associated with pro-inflammatory action (i.e., T_H1 and T_H17) and the cells that suppress that action (T-reg).

This inability of postpartum depressed and postpartum psychotic women to mount a physiological T-cell immune activation in the postpartum period suggests a defect in the T-cell system. Indeed, older research on functional T-cell parameters (such as lymphocyte stimulation assays) delivers evidence for such a defect in the T-cell system intrinsic to those with a major mood disorder (Toben and Baune, 2015). We reported that subjects with a major depressive episode (outside the postpartum period) were characterized by decreased serum levels of the T-cell growth factors IL-7 and sCD25 and by mildly reduced levels of T-helper and T-regulatory cells (Grosse et al., 2016). Snijders and colleagues similarly reported reduced levels of T cells and T-helper cells in children of a bipolar parent (at high risk for a mood disorder) from adolescence to young adulthood (Snijders et al., 2016), and another study from the same group showed that the familial liability to develop bipolar disorder determined the reduced levels of T cells (Snijders et al., 2019). Two other groups also confirmed shifts in the T-helper populations, with T regulatory cells decreased and T_H17 and T_H2 cells increased in bipolar disorder (Becking et al., 2018; Vogels et al., 2017) in contrast to the *decreases* in T_H17 cells characteristic for unipolar depression (Becking et al., 2018). In sum, there is ample evidence that T-cell defects mark mood disorders, and our data here indicate that this pattern extends to postpartum depression as well.

Of course, merely establishing a connection between T-cell defects and postpartum affective disorders does not significantly advance our science about either the results or the causes of such disorders. If women with PPD have T cell defects, are they in fact more vulnerable to postpartum infections? Is their tolerance to paternal antigens in future pregnancies lest robust than that of healthy women? To our knowledge there are no data supporting a higher infection rate or an increased spontaneous abortion rate in subsequent pregnancies for women suffering from a postpartum depression (though a higher infection rate has been described in major depressed individuals in general) (Kohler-Forsberg et al., 2019). T cell defects might also have substantial effects on brain development and white matter integrity. Poletti and colleagues (Poletti et al., 2017) found the percentage of circulating T_H17 cells to correlate positively with white matter integrity, particularly in fiber tracts connecting the forebrain with the limbic system, in both healthy controls and bipolar depressed subjects. The frequency of circulating T-regulatory cells correlated positively with white matter tract disruption in these areas and to lower neuronal responses to negative versus positive morally tuned stimuli in the right dorsolateral prefrontal cortex of bipolar depressed subjects.

With regard to the origin of the T-cell defects in subjects with mood disorders, particularly in the postpartum, a few putative mechanisms come to mind. Tryptophan is an essential growth factor for T cells, and reduced tryptophan levels are a hallmark of mood disorders. In a previously published paper we showed reduced tryptophan levels in both postpartum depression and postpartum psychosis (Veen et al., 2016), and it is tempting to speculate that these reduced tryptophan levels are related to the T cell defects. Other groups have had similar findings (Duan et al., 2018; Teshigawara et al., 2019). It may also be that non-depressed people have the ability to buffer a decrease in tryptophan that occurs for all women after childbirth, as increases in cortisol spur immune activity that downregulates the metabolism of tryptophan into serotonin (Duan et al., 2018). Substantial work is clearly needed on the connection between these T-cell defects and other findings showing increases in inflammatory activity postpartum, measured primarily in cytokines (Brann et al., 2017; Osborne and Monk, 2013) – we may need to look more at function of different immune cell populations than at number. In this case, the population may prove illuminating, as our sample was limited to women who developed new-onset symptoms in the postpartum and many cytokine studies include women who were or may have been depressed in pregnancy as well.

Another possibility is the interaction with pregnancy hormones. Sex steroids and prolactin are known to influence T-cell growth and differentiation (Recalde et al., 2018), and altered levels of these hormones have been suggested in at least some studies of postpartum mood disorders, though evidence is mixed (McEvoy and Osborne, 2019; Osborne et al., 2017; Schiller et al., 2015). Studies that link endocrine alterations to those of the immune system in postpartum mood disorders do not yet exist.

Our study has a number of limitations. While we were able to include reasonably large control groups, the sample of women with postpartum depression is quite small, given the stringent inclusion criteria (postpartum onset, severe depression, largely antidepressant-free). Because we wanted to restrict to postpartum onset, our group of cases does not match the DSM-V definition, which requires onset during pregnancy *or* within 4 weeks postpartum. We may, therefore, have missed small differences among groups that would have been evident in a larger population (but thus also avoided the heterogeneity common to studies on PPD that have less strict inclusion criteria). In addition, we did not have sociodemographic details other than weight and BMI for our non-postpartum healthy controls, so it is possible that some of our results reflect differences between groups that are affected by these characteristics. Only one of our subjects was taking antidepressant medication, so we were unable to control for this variable in our analyses. Other limitations may come from possible differences among samples in the amount of time spent in storage (though we are aware of no literature that addresses whether such differences actually exist), or from the timing difference in the blood draw between groups. We are reassured on the latter point, however, because we controlled for this difference in our analyses, and limited previous literature actually supports an increase in T-helper activity across the postpartum period, which would mean that our PPD subjects (who had the later blood draw) should show HIGHER and not LOWER activity than our healthy subjects (Watanabe et al., 1997). In addition, our T-cell data do not represent absolute counts per ml of blood but are instead relative to numbers of

PBMCs and lymphocytes; future studies would benefit from measuring the absolute numbers of leukocytes per ml blood at the time of testing.

Despite these limitations, our work adds to the small but growing number of investigations of immune alterations in perinatal mood disorders that attempts to reach beyond measures of peripheral cytokines to look at other immune system defects that may play an etiological role in these devastating illnesses. Our clear results showing that healthy postpartum women show a rebound of T-cell activity, particularly in pro-inflammatory activity (T_H1) and compensatory mechanisms (Treg) is consistent with consistent with previous literature on pregnancy. Our finding that postpartum depressed women do not mount this response adds to our previous similar results in a population of women with postpartum psychosis, and adds to the growing body of literature indicating that T-cell dysregulation may be an important feature of mood disorders. Future research characterizing these differences in larger populations, and extending into different classes of immune cells, will be instructive

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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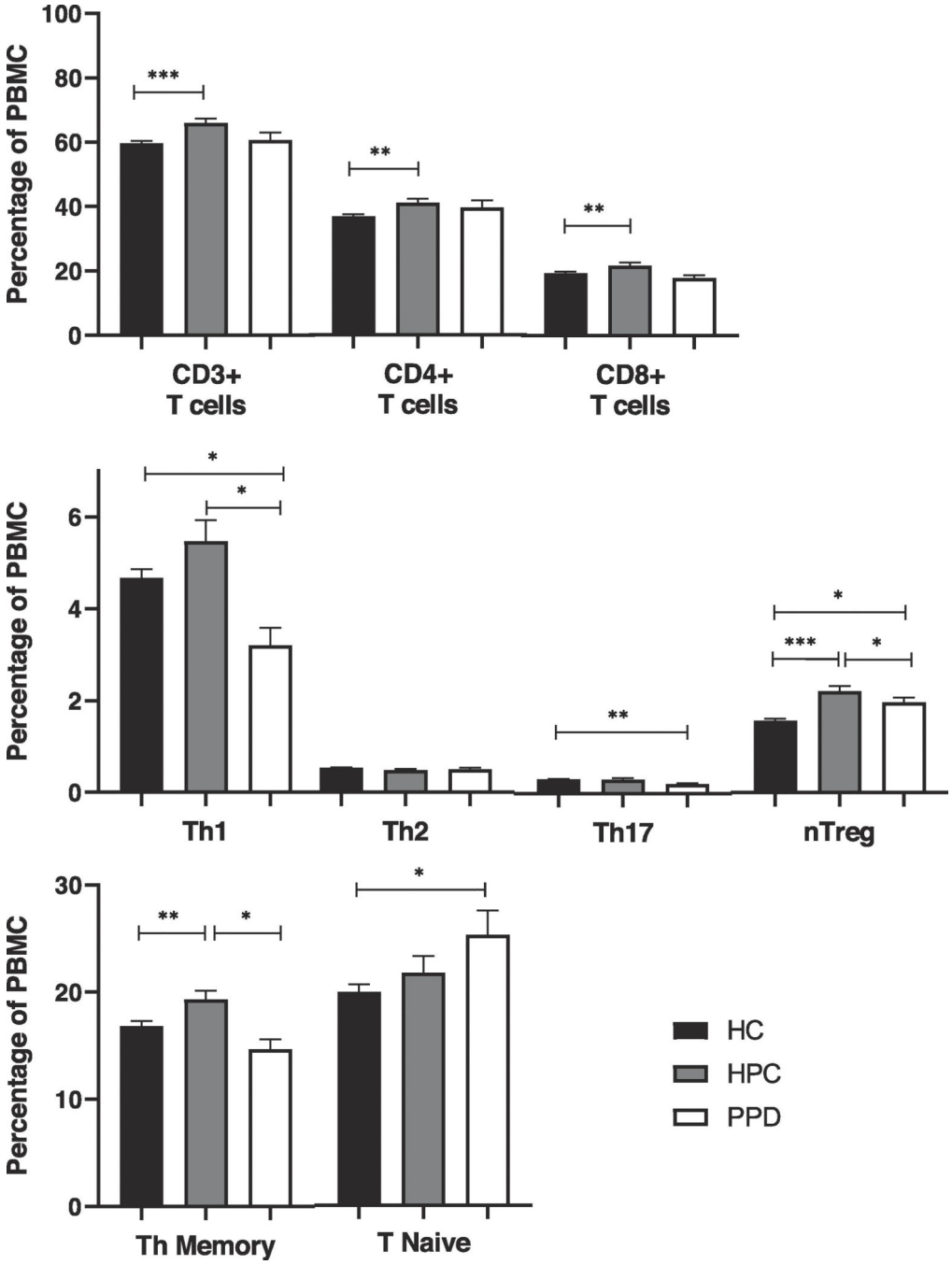
Highlights

- Little is known about T-cell functioning in postpartum depression (PPD)
- We compared women with postpartum depression to two groups of healthy controls
- Healthy postpartum women had higher mean T-cell counts than non-perinatal women
- Women with PPD failed to show this physiological enhanced T-cell activity



Percentage of T-cells and T-cell subset populations (as percentage of lymphocytes) in three populations: postpartum depression (PPD, N=21, in white); healthy postpartum controls (HPC, N=39, in gray); and healthy non-postpartum controls (HC, N=114, in black).

Table 1.

General and obstetric characteristics of subject with first-onset postpartum depression (PPD), healthy postpartum controls (HPC), and healthy non-postpartum controls (HC)

	HC (n=114)		HPC (n=39)		PPD (n=21)		Difference between HPC and PPD
	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Age (years)	30.25	(.61)	33.00	(.67)	32.09	(1.12)	0.492
Weight	67.88	(.92)	71.55	(1.67)	74.66	(2.81)	0.322
BMI	23.12	(.27)	23.88	(0.50)	26.97	(0.96)	**0.029**
Blood withdrawal, days postpartum			30.97	(2.84)	61.00	(8.50)	**<0.001**
			n	%	n	%	
Dutch ethnicity			35/39	89.7%	17/20	85.0%	0.594
Education beyond high school			37/39	94.9%	14/19	73.7%	**0.020**
Married/ cohabiting			36/39	92.3%	18/20	90.0%	0.763
Primiparity			24/39	61.5%	16/21	76.2%	0.251
Primigravidity			22/39	56.4%	13/21	61.9%	0.681
Caesarean section			5/39	12.8%	5/21	23.8%	0.276
Vacuum extraction			4/39	10.3%	3/21	14.3%	0.687
Breastfeeding			28/39	71.8%	1/21	4.8%	**<0.001**
Medication use					12/21	57.1%	

HC = healthy non-postpartum controls, HPC = healthy postpartum controls, PPD = patients with postpartum depression

Table 2.

Proportions of T cell subsets across all three groups.

Percentage of Peripheral Blood Mononuclear Cells (PBMCs)	**HC N=114**	**HPC N=39**	**PPD N=21**	**HC vs. HPC HC**		**HC vs. PPD**		**HPC vs. PPD**	
	M (SEM)	**M (SEM)**	**M (SEM)**	**p value**[1]	**Cohen's *d***	**p value**[1]	**Cohen's *d***	**p value**[2]	**Cohen's *d***
CD3+ T cells	59.74 (0.62)	66.02 (1.35)	60.65 (2.36)	**<0.001**	0.83	0.458	0.10	0.122	0.56
CD4+ T cells	36.87 (0.65)	41.02 (1.30)	39.65 (2.20)	**0.007**	0.55	0.431	0.33	0.260	0.15
CD8+ T cells	19.35 (0.42)	21.68 (1.06)	17.85 (0.91)	**0.002**	0.41	0.994	0.35	0.428	0.70
T_H1	4.67 (0.19)	5.47 (0.46)	3.20 (0.38)	**0.032**	0.33	**0.031**	0.81	0.069	0.99
T_H2	0.53 (0.01)	0.48 (0.03)	0.50 (0.04)	0.082	0.30	0.390	0.19	0.826	0.12
T_H17	0.31 (0.01)	0.31 (0.03)	0.21 (0.02)	0.896	0.01	**0.006**	0.57	0.365	0.55
T reg	1.57 (0.04)	2.21 (0.11)	1.96 (0.11)	**<0.001**	1.09	**0.030**	0.82	**0.044**	0.42
T_H memory	16.83 (0.48)	19.32 (0.81)	14.68 (0.94)	**0.009**	0.49	0.066	0.46	**0.017**	0.99
T_H naive (*calculated*)	19.91 (0.69)	21.70 (1.53)	25.22 (2.27)	0.333	0.21	**0.045**	0.60	0.356	0.36

[1] Adjusted for BMI

[2] Adjusted for BMI, postpartum day of blood draw, and educational level.