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Suppression of Natural Killer Cell Cytotoxicity in Postpartum Women: Time Course and Potential Mechanisms

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Abstract

Little is known about the recovery of the immune system from normal pregnancy and whether the postpartum period is a uniquely adapted immune state. This report extends previous observations from our group of decreased natural killer (NK) cell cytotoxicity in the postpartum period. NK cytotoxicity was measured from 1 week through 9 months postpartum. In addition, NK cytotoxicity was assayed in the presence or absence of pooled plasmas collected from either postpartum or nonpostpartum women. Samples of cells were stained for inhibitory receptors and analyzed by flow cytometry.

NK cytotoxicity remained decreased in postpartum women compared to controls through the first 6 postpartum months, returned to normal levels by 9 months and remained normal at 12 months. NK cytotoxicity during the first 6 months was further inhibited by the addition of pooled plasma to NK cultures from postpartum women, but the addition of pooled plasma from the control group did not affect that group's NK cultures. There were differences in inhibitory receptor staining between the two groups, with decreased CD158a and CD158b and increased NKG2A expression on postpartum NK cells during the first 3 postpartum months. These data suggest that NK cytotoxicity postpartum inhibition lasts 6 months and is influenced by unidentified postpartum plasma components. The effect may also involve receptors on NK cells.

Keywords

NK cytotoxicity; postpartum inhibition; plasma effects; flow cytometry; human leukocyte antigen-G

Natural killer (NK) cells are large granular lymphocytes normally comprising 5-15% of peripheral blood lymphocytes. Peripheral NK cells are identified as CD3 negative and CD16 and CD56 positive (Cooper, Fehniger, & Caligiuri, 2001). The majority (around 90%) of peripheral NK cells are classified as CD56^{dim} (Caligiuri, 2008). NK cells are considered part of the innate immune system and lyse transformed, infected, and allogeneic cells without requiring prior sensitization (Konjevic et al., 1995). They also release type 1 cytokines such as interferon (IFN)- γ and tumor necrosis factor (TNF)- α and regulate the adaptive immune system. Regulation of NK cytotoxicity is complex and involves a balance between activating and inhibitory receptors and interaction with cytokines and major histocompatibility (MHC) receptors on target-cell surfaces (Bryceson & Long, 2008). Normal levels of MHC molecules on target cells produce inhibition of NK effector action by engaging as ligands to the NK inhibitory receptors. When cells have decreased MHC or lack self-MHC, the activating receptors produce killing through release of cytotoxic molecules such as perforin.

Peripheral CD56^{dim} cell numbers are reduced in pregnancy (Kraus et al., 2012) and show diminished function (cytotoxicity and cytokine secretion; Borzychowski, Croy, Chan, Redman, & Sargent, 2005; Gregory, Lee, et al., 1985; Gregory, Shah, Lee, Scott, & Golding, 1985; Kraus et al., 2012). Kraus et al. followed 50 women prospectively across pregnancy and found both numbers and functions of NK cells to be reduced. It is presumed that the peripheral NK cell decrease in pregnancy is one of the multiple mechanisms that allow fetal semi-allogeneic cells to escape attack by the maternal immune system. One of several mechanisms for NK cell downregulation in pregnancy is interaction of NK cells with cells bearing human leukocyte antigen (HLA)-G or with soluble HLA-G (sHLA-G). HLA-G, which is present on fetal and cytotrophoblast cells and is shed in a soluble form, acts through receptors on NK cells to induce apoptosis and inhibit proliferation and cytotoxicity (LeMaout, Zafaranloo, Le Danff, & Carosella, 2005).

The postpartum period may reflect the physiological alterations that occur in pregnancy for a period of time. Through upregulated innate immune mechanisms, a state of controlled sterile inflammation is required in early and late pregnancy for successful implantation and labor and delivery. This state may be a response to circulating trophoblastic debris, which is common in pregnancy (Chamley, Chen, Ding, Stone, & Abumaree, 2011). The orchestration of immune balance during these periods is toward a downregulated type 1 (cellular) and upregulated type 2 (humoral) immune state, along with aspects of exaggerated inflammation (Challis et al., 2009; Mor, Cardenas, Abrahams, & Guller, 2011).

NK cells are thought to play a role in regulating the immune system in pregnancy (Borzychowski et al., 2005). Nevertheless, peripheral NK cells (in contrast to uterine NK cells) do seem to be diminished in number and less active in pregnancy. In an earlier cross-sectional study of 200 women at 4–6 weeks postpartum, we found that the NK cell number was reduced compared to controls (Groer et al., 2005). With regard to NK functional

activity, we previously reported that NK cytotoxicity was reduced through 6 postpartum months in a small sample of 39 women being measured in a larger study of postpartum immunity (the parent study; Groer, El-Badri, Djeu, Harrington, & Van Eepoel, 2010). We conceptualized this reduced NK cytotoxicity in the postpartum period to be a continuation of pregnancy-induced NK cytotoxicity suppression, proposing that it could possibly facilitate tolerization of fetal microchimeric cells to survive and establish themselves in tissue niches (Groer, Manion, Szekeres, & El-Badri, 2011).

The need for further investigation of the role of NK cells during the postpartum period is related to the essential function of these cells in immune surveillance. The suppression of function must have a significant benefit for maternal health that overrides the need for the normal NK cytotoxic balance. Clarifying the length of time that NK cells are suppressed and the molecular nature of the suppression will illuminate this phenomenon and provide for potential clinical interpretations. When we received additional funding in the parent study, we extended the follow-up period to 9 and 12 months in participants we were continuing to recruit into the parent study and were able to design experiments to investigate potential mechanisms of NK suppression.

In this continuation of work we specifically asked the following questions:

1. What is the length of postpartum NK suppression?
2. Is the source of suppression one or more soluble plasma components such as cytokines or unique biomarkers of this reproductive phase such as hormones or sHLA-G, or is suppression accomplished through NK inhibitory receptor expression?

Materials and Method

Participants

The original sample of 39 women, about whom we previously published findings, were early participants in the parent study. We have now completed data collection in that study and can report on 107 women followed across 6 months, with subsets followed for 9 ($n = 24$) and 12 ($n = 10$) months. At 1 week postpartum there were 93 participants, and at 1 month, 107. Thereafter we had data on 103 at 2 months, 89 at 3 months, 89 at 4 months, 84 at 5 months, and 79 at 6 months. The drop-off in participants was related mostly to women moving to different locations and becoming unavailable, with a few being lost to follow-up because their contact information changed and we could not locate them. Occasionally participants were too anemic for blood draw (hematocrit less than 34%) or there was no or an inadequate blood sample provided. Very occasionally we lost data due to laboratory errors.

All women gave informed consent to participate, and the university's Institutional Review Board approved the study. We recruited the women for the postpartum study during prenatal visits in several large private and public prenatal clinics between 16 and 25 weeks of pregnancy. Exclusion criteria included HIV diagnosis, drug addiction, autoimmune disease, thyroid disease, medications that could affect immunity, extreme thinness and in vitro

fertilization. The parent study investigated postpartum thyroiditis (PPT), and 37 women were positive for the thyroid peroxidase (TPO) antibody but never developed PPT. Any women who developed PPT at any time in the postpartum period were not included in the current analyses as the inflammatory nature of this disease might have confounded the findings. Repeated-measures, mixed-model analyses of variation (ANOVAS) of lytic units (LUs) comparing TPO-positive and -negative women showed no statistically significant difference in LUs by group over time, so we included both the TPO-negative and TPO-positive women without PPT in the present analyses. Nonpostpartum, age-matched women ($n = 16$) provided blood samples that we used in several of the assays (controls).

Assessments and Measures

Isolation of peripheral blood mononuclear cells (PBMCs) and NK cell cytotoxicity assay (NKCA)—A research nurse drew 15 ml of blood from participants at monthly home visits. The blood was collected in heparinized tubes and brought to the lab in a cooler within 2 hr for immediate processing. PBMCs were isolated by Ficoll-Histopaque (Sigma-Aldrich) density gradient centrifugation, washed twice in RPMI-1640 media (Fisher) with 10% fetal calf serum and 0.05 mg/ml gentamycin (Fisher). Plasma was separated and frozen in aliquots at -80°C for cytokine, hormone, and sHLA-G measurement. Aliquots of PBMCs were preserved in 10% dimethyl sulfoxide (DMSO) and stored in aliquots in liquid nitrogen for later phenotyping by flow cytometry. For the NKCA, additional aliquots of fresh, washed PBMCs (4×10^6) were pre-incubated in RPMI-1640 media overnight with 5 units/ml of recombinant interleukin-2 (rIL-2) in an incubator set at 37°C with 5% CO_2 added to the incubator's atmosphere. Without this IL-2 pre-incubation, the cytotoxicity of these postpartum NK cell cultures was extremely low or non-existent. The PBMCs were then co-cultured the next day with 100 uCi Cr^{51} -labeled K562 cells for 5 hr for the NKCA, as previously reported (Groer et al., 2010). NK cytotoxicity data are reported as LUs or as percent cytotoxicity at the 50:1 effector:target (E:T) ratio, depending on the experiment. Not all participants' samples had an NKCA done at each monthly time point because of the volume of blood needed for multiple other assays in the parent research study, lack of a sample, or viability issues. Viability of 80% or higher in the NKCA cultures was determined to be optimal. The participants who had samples available for NKCA at each time interval ranged from 58 women at Week 1 to 79 at Month 3 and 63 at Month 6. In addition four samples were extreme outliers with LUs 3–16 times the standard deviation, and they were removed from analysis.

Pooled plasma experiments—Equal aliquots of randomly selected plasma samples collected at postpartum Month 1, 2 or 3 were pooled (total number of samples = 34). These aliquots were all from different participants and were therefore a representative pooled sample. Pooled plasma was used rather than autologous plasma as the PBMCs had been washed several times before incubation and the goal of the experiment was to determine if a uniform set of postpartum plasma components would influence lytic activity of these cells. Pooling multiple plasmas provided the best opportunity to measure this effect. Experiments were then done in which 50 μL s of this pooled postpartum plasma were added to NKCA at each postpartum time to compare percent cytotoxicity at the 50:1 E:T ratio in cultures with and without added pooled plasma. NK cells from the same participant ($N = 20$) were

analyzed prospectively in these experiments (total number of experiments = 74) at the separate postpartum times. To ensure that any effect was not due to volume or osmotic influences, the experiments were volume controlled and experiments to test the effect of heat-inactivated plasma were done to ensure that any plasma effect was due to biological molecules rather than other (e.g., pH, osmotic) effects. Experiments were also done in the same manner on NK cultures from nonpostpartum participants ($n = 16$), adding either postpartum or control pooled plasma to the cultures and measuring cytotoxicity at 50:1, in order to determine if any effect observed was unique to postpartum cultures.

Flow cytometry—PBMCs from 33 postpartum women collected at Months 1, 2 or 3 and 16 age-matched controls were labeled with a series of fluorescent antibodies to NK-cell inhibitory receptors. NK cells were labeled with monoclonal antibodies to CD16 and CD 56 (Biolegend), and then the following fluorescent monoclonal antibodies were used to label the inhibitory receptors: CD158a and CD158b, CD94, NKG2A (Miltenyi Biotec). Freshly isolated 1×10^6 PBMCs were washed three times in RPMI and then incubated for 30 min at 4° C with appropriate conjugates of the monoclonal antibodies, combined to allow for eight-color flow cytometry (FACS) analysis. These cells were then fixed in paraformaldehyde prior to analysis and kept in the cold until they were read within 24 hr. CD3- cells were gated on expression of both CD16 and CD56 markers. The percentages of cells with the various surface markers were measured on a BD LSRII flow cytometer and analyzed with Flow Jo software. They were expressed as percentages of the parent population of CD3-, CD56+ and CD16+ cells.

Plasma cytokines and hormones—Cytokines (IFN- γ , IL6, IL10, IL4 and TNF- α) were measured in the plasma samples at each monthly time point by multiplexing with Millipore kits (St. Louis) and analysis in a Luminex-200. Estradiol, progesterone, and prolactin (ALPCO) and cortisol (DRG) were all measured by ELISA at Week 1 and then again at Months 1, 3, and 6 postpartum. Soluble HLA-G1 and G5 were also measured by ELISA (BioVendor) at these time points ($n = 53$ at Week 1, $n = 79$ at Month 1, $n = 69$ at Month 3, $n = 44$ at Month 6). Not all participants had ELISAs done at every time point for a variety of reasons such as no blood sample because of anemia, inadequate blood sample for all the measures in the study, or missed visit. These ELISAs were performed according to kit directions and had intra-assay coefficients of variation less than 10%.

Statistical Analyses

We assumed that 30 postpartum women would contribute an average of six blood samples over time to the analyses coupled with 16 single measurements from the normal controls. With a two-sided type error rate of 0.05, the study would provide 80% power to detect modest-to-large effect sizes of 0.58, 0.66, and 0.72 assuming within-subject (postpartum women) correlations of 0.2, 0.4, and 0.6, respectively.

CD158a, CD158b and sHLA-G data were logarithmically transformed, as they were positively skewed. T-tests were done to compare the postpartum sample LUs to controls for every time point. T-tests were also done to compare groups on inhibitory membrane markers. Pearson correlation coefficients were computed to analyze relationships between

sHLA-G, LUs, and membrane markers. Paired t-tests were computed to compare cytotoxicity in the absence and presence of pooled plasma.

Results

Participants

The mean age of women in the postpartum group was 28.7 years and their mean parity was two. The majority were married (62%); 60% had annual incomes greater than \$40,000, while 10% had incomes under \$5000; 40% were college educated. The racial/ethnic composition of the postpartum group was as follows: 43% Caucasian, 29% Hispanic, and 14% African American, with the remainder comprising other ethnicities or undisclosed. Approximately one-third (33%) had delivered by Caesarean section, with the remainder delivering vaginally. The majority had breastfed at least some of the time in the postpartum period. None of these demographic variables influenced NK cytotoxicity across the postpartum period. In other words, there were no significant correlations between socioeconomic variables and LUs, and t-tests comparing LUs between groups based on type of delivery or breastfeeding were nonsignificant.

The controls were volunteers (mainly students and staff from the college) who were between the ages of 18 and 45 years. We collected very little information from them, other than determining their age and race and that they were not experiencing stressful life situations.

Time Course

Figure 1 depicts the comparison between NK cell cytotoxicity in postpartum women across 9 months of the postpartum period and in control women. There was a range in the number of assays done for each time point, from 58 to 79, except for 21 at Week 1 and 24 at Month 9. The findings at 9 and 12 months were identical, though we had a smaller sample at 12 months ($n = 10$). The difference between the LUs for the control and postpartum groups was significant at all time points through 6 postpartum months, with t -values ranging from 2.9 to 4.1 and p -values all $< .001$ for the first 5 months; $t = 1.0$, $p = .05$ at 6 months; $t = .87$, $p = .39$ at 9 months; and $t = 1.27$, $p = .12$ at 12 months.

Postpartum Plasma Effects

The addition of heat-inactivated plasma had no effect on NK cytotoxicity, suggesting that any effect we observed with the addition of pooled plasma would be attributable to biological molecules in that plasma. For these experiments we used the percent lysis at the 50:1 E:T ratio in the NKCA rather than LUs due to constraints of cell numbers. The 50:1 E:T ratio produced the greatest cytotoxicity in our assay and was highly correlated with total LUs ($r = .70$, $p < .001$). Comparisons of cytotoxicity in the presence and absence of pooled postpartum plasma across the postpartum period showed a significant suppression of cytotoxicity with the addition of pooled plasma. The average percent cytotoxicity across 6 months for the samples with added pooled plasma was 15.4 ± 1.6 S.E.M. compared to the control cytotoxicity of 26.6 ± 1.4 S.E.M. ($t = 10.3$, $df = 73$, $p < .001$; Figure 2). Addition of pooled postpartum plasma to nonpostpartum age-matched control women's NK cell cultures ($n = 16$) did not affect cytotoxicity. The cytotoxicity at an E:T ratio of 50:1 was 10.7 in

control wells (no added plasma) and 10.01 when pooled postpartum plasma was added (nonsignificant). These data suggest a that postpartum NK cells are uniquely responsive to plasma components.

Plasma cytokines were not correlated with NK cytotoxicity at any time point. The sHLA-G values across the postpartum period remained stable (29–36 U/ml), dropping slightly at Month 6 (Figure 3). There was a significant inverse correlation between LUs and sHLA-G levels at Week 1 ($r = -.34$, $p = .02$) and Month 3 ($r = -.32$, $p = .02$), but the correlation was not statistically significant at the two other measurement times. There was a significant correlation of sHLA-G concentration with NKG2A expression on postpartum NK cells ($r = .57$, $p < .02$).

Plasma concentrations of estradiol, progesterone, prolactin and cortisol measured at Week 1 and Months 1, 3 and 6 showed no correlations with LUs at any time point. Breastfeeding women did not differ from formula-feeding women in LUs across the postpartum period.

Flow Cytometry

Phenotyping of cells by flow cytometry showed that the percentage of NK cells did not change significantly over the first 3 postpartum months (data not shown). The average percentage of NK cells in the total lymphocyte population in 33 postpartum samples measured at Months 1, 2 and 3 was 9.3% (range 0.9–39; $SD = 4.7$). The measures at Months 1 and 2 were nearly identical (8.9% and 8.2%), but the NK percentage did rise to over 11% at 3 months. Control NK percentage ($n = 16$) was 7.2 (range 1.5–21.2). We analyzed flow cytometric phenotyping of inhibitory receptors of NK cells from postpartum women compared to those from nonpostpartum age-matched women who served as controls (Figure 4). These cells were preserved from the initial washed PBMC sample in liquid nitrogen and had not undergone any manipulation or culturing with rIL2. Inhibitory receptors measured were the lectin-type receptors CD94 and NKG2A and the killer immunoglobulin-like receptors (KIRs) CD158a and CD158b. There was a significantly lower percent staining of CD158a ($t = -5.3$, $p < .001$) and CD158b ($t = -2.5$, $p < .017$) receptors in the postpartum NK cells compared to the control NK cells. The percent NKG2A staining of NK cells was significantly higher in postpartum women compared to controls ($t = -2.55$, $p = .01$). CD94 expression was not significantly different ($t = .8$, $p = .4$) between the groups, nor were the percentages of total NK cells ($t = .99$, $p = .3$) in this sample.

Discussion

There are many potential pathways through which NK cytotoxicity could be suppressed in postpartum women. Plasma components including cytokines and hormones might conceivably play a role during this reproductive phase, but in this study there were no relationships between these plasma components and cytotoxicity. Other mechanisms could include activation of the KIR receptors, inhibition or reduced expression of activation markers, blocking of lysis, inhibition of cytokine secretion, or interruption of NK–target conjugate formation (Gan, Zhang, Solomon, & Bonavida, 2002).

Data from the present study clearly show that NK suppression extends for several months postpartum and that plasma components and possibly NKG2A receptors are involved in this suppression. The experimental results suggest that only postpartum, and not nonpostpartum control, NK cells were uniquely responsive to the addition of pooled postpartum plasma components. Plasma levels of sHLA-G were inversely correlated with LUs in the early postpartum period, suggesting that it may be an NK-cell suppressive molecule. This hypothesis is further supported by the relationship between NKG2A expression and sHLA-G levels in the plasma. sHLA-G may still be present in postpartum plasma as the result of the shedding of cytotrophoblastic cells in pregnancy or as a marker of fetal microchimerism. The HLA-G gene is low polymorphic, and its expression in fetal cells is accompanied by a lack of classical Class I HLAs and Class II antigens. This state leads to an inability of maternal T and NK cells to recognize fetal cells as foreign (Rebmann, Regel, Stolke, & Grosse-Wilde, 2003). sHLA-G can be detected in healthy women (mean of 20.7 $\pm$ 2.1 ng/ml; Pistoia, Morandi, Wang, & Ferrone, 2007) and is increased in pregnancy, as we also found in this postpartum sample. Other sources of HLA-G include the thymus, cornea, nail matrix, pancreas, erythroid and endothelial precursor cells, and monocytes (Fainardi et al., 2011). The NK receptors thought to bind to HLA-G include KIR2DL4 (which is difficult to measure) and possibly CD94/NKG2A (which may respond to peptides derived from HLA-G and presented by HLA-E; Apps, Gardner, & Moffett, 2008). There was not a consistent relationship between LUs and sHLA-G across the postpartum period, so it is unlikely that sHLA-G was the sole source of plasma suppression of cytotoxicity.

NKG2A forms a dimer with CD94 and acts as an inhibitor of NK cytotoxicity. We found that NKG2A was markedly increased in postpartum women compared to control women. CD94 was not upregulated in postpartum women, but this may have been due to the fact that CD94 also dimerizes with other molecules, some of which are activating receptors. Recently investigators reported that the NKG2A–CD94 complexes potently suppress uterine NK cell cytotoxicity (Le Bouteiller et al., 2011). NKG2A–CD94 binds to HLA-E on cells, and HLA-E, like HLA-G, is a nonclassical, low polymorphic Class I MHC molecule. Like HLA-G, it is also highly expressed by cytotrophoblast cells in pregnancy (Ishitani, Sageshima, & Hatake, 2006).

Expression of CD158a and CD158b was decreased in NK cells from postpartum women compared to those from controls in the present study. These KIRs react with classical Class I HLA molecules (HLA-A, HLA-B, and HLA-C). There may be differential patterns of receptor expression on postpartum NK cells that respond to inhibitory or activating signals. The decreased expression of CD158a and CD158b we observed in the freshly stained postpartum NK cells may be related to an absence of IL-2 in the media. IL-2 is needed to increase the expression of these inhibitory receptors (Marras, Bozzano, & De Maria, 2011). We did note that postpartum cells had an extreme dependence on IL-2 and were essentially nonfunctional in the absence of IL-2.

Remarkably, while pooled postpartum plasma consistently and significantly increased inhibition of NK cytotoxicity in the NK cultures of postpartum women, it did not have any effect on nonpostpartum control women's NK cultures. Therefore it seems likely that both plasma factors and NK-receptor functions are involved in the NK cytotoxicity suppression

of the postpartum period. This proposition suggests that postpartum NK cells have unique properties compared to NK cells from nonpostpartum women, set apart by as-yet-unknown mechanisms.

It would be instructive to compare our findings in the present study with studies of NK cytotoxicity in pregnant women, since it is reasonable to assume that the postpartum period may extend pregnancy effects. We do know that cytotoxicity and number of peripheral CD56 cells are decreased in normal pregnancy, that these cells play a role in orchestrating pregnancy-associated type 2 shifting, and that increases in the number of NK cells are associated with spontaneous abortion (Lashley et al., 2011; Lu et al., 2011). The mechanism by which this decline in number and cytotoxic activity of peripheral NK cells occurs is not well described. Research is now focused on uterine NK cells, which differ in multiple ways from peripheral NK cells. Peripheral NK cells are more cytotoxic and less secretory than the CD56^{bright} uterine NK population. The low cytotoxicity and the interaction of plasma components with possibly upregulated inhibitory receptor NKG2A suggests that ligands, including sHLA-G, in plasma may be involved in NK suppression in the postpartum period through inhibitory receptors. Researchers have previously identified several plasma components as inhibitors of NK cytotoxicity against K562 cells (Rolin, Sand, Knudsen, & Maghazachi, 2010). Further work is needed to identify unique components of postpartum plasma.

Limitations and Implications for Future Research

The research presented here is preliminary and exploratory. We were constrained by availability of adequate cell numbers and available samples. Other inhibitory as well as activating receptor phenotyping is warranted in future work. We acknowledge that the staining results from unmanipulated cells may be very different than those from cells after rIL-2 pre-incubation. It was difficult to obtain enough cells to stain after IL-2 incubation and still have enough for the NKCA, as there was always some cell loss with each incubation. Additional aspects of NK cytotoxicity such as perforin and granzyme activity would be of interest. Isolation of the unique components of plasma that increase the suppression of NK cytotoxicity and further investigation of the role of sHLA-G in NK function are also warranted.

Identification and isolation of these components, along with an increased understanding of their pathways of interaction with NK cells, may prove useful in translational medicine. Understanding the immune system of healthy postpartum women may reveal mechanisms that can ultimately be informative about or lead to new treatments in disease states. It seems obvious that healthy postpartum women are not particularly at risk even in the presence of significant NK cytotoxicity suppression, and the physiology associated with this surprising finding bears additional scrutiny.

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Figure 1.

Natural killer (NK) cell cytotoxicity (measured in lytic units) in women across postpartum months compared to controls. Bars represent means, and error bars are standard errors of the mean. NK cell cytotoxicity was significantly lower in postpartum women than in controls through 6 months postpartum. * $p = .05$. ** $p = .01$. *** $p = .001$. The difference between groups was nonsignificant (NS) at 9 months and at 12 months (data not shown).

Figure 2. Natural killer (NK) cell cytotoxicity at 50:1 effector:target (E:T) ratio in postpartum NK assays ($n = 34$) with and without the addition of pooled postpartum plasma. Error bars are standard errors of the means.

Figure 3.
Soluble human leukocyte antigen (sHLA)-G staining across the postpartum period.

Figure 4.

Inhibitory-receptor staining of postpartum natural killer (NK) cells ($n = 31$) compared to cells from age-matched nonpostpartum controls ($n = 10$). Error bars represent standard errors of the means. CD158a and CD158b were significantly lower ($p = .001$ and $.017$, respectively), and NKG2A significantly higher in postpartum compared to controls ($p = .03$).