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Immune Responses in Mothers of Term and Preterm Very-Low-Birth-Weight Infants

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Differences in the levels of immune cell subsets present in peripheral blood have been demonstrated based on sociodemographic factors such as age and race. Postpartal women, who are recovering from the immune changes that are concomitant with pregnancy, have lymphocyte and monocyte values that differ from other populations. A subgroup of postpartal women, mothers who deliver preterm very-low-birth-weight (VLBW) (≤1,500 g) infants, may have further differences in values of immune cell subsets and in immune functioning either because of hormonal factors or lifestyle changes or because of the stress they experience after their infant's birth and for the first few months of infant caretaking. This study examined anxiety, depression, and immune cell phenotypes in 30 mothers of VLBW infants and in 30 mothers of healthy term infants over the first 4 postpartal months to determine if mothers of preterm VLBW infants differed from mothers of healthy term infants in psychological and immunologic parameters. Additionally, lymphocyte proliferation and natural killer cell functional assays were performed in a subset of mothers. Mothers of VLBW infants had increased anxiety and decreased lymphocyte proliferation compared to mothers of term infants. When lymphocyte and monocyte subsets were compared over time between the two groups of mothers differences were found in CD8, CD20, $CD3^-/CD56^+$, CD14, and HLA class II Ia on monocytes. Mothers with high-fat diets had lower percentages of some monocytes (CD14), lymphocytes ($CD4^+/CD45RA^+$), and natural killer cells ($CD3^-/CD57^+$) during the first 4 postpartal months.

Sociodemographic factors such as age, gender, and race are known to contribute to differences in the levels of monocyte and lymphocyte subsets present in peripheral blood (31, 36). Not surprisingly, therefore, postpartal mothers of term infants, who are recovering from immune changes concomitant with pregnancy, differ from men and nonpostpartal women in immune cell phenotypes during the first 4 postpartum months (18). A subgroup of women, mothers who deliver preterm (<37-week-gestation), very-low-birth-weight (VLBW) (≤1,500 g) infants may, because of lifestyle or hormonal changes or stress, have further differences in the levels of immune cell subsets and in immune functioning than do mothers of healthy term infants.

Stress may influence both the quantity and functioning of immune cell subsets (20). Anxiety and depression have also been related to decreases in the number of circulating monocytes (20) and natural killer (NK) cells (10, 11) in a wide range of circumstances (widowers [2, 5], pregnant women [21], divorcees [27], and caretakers of elderly Alzheimer's patients [26, 28]). The initial unstable health of VLBW and preterm infants and the long hospitalization required before they are able to go home has been related to increased anxiety and depression for their mothers both during infant hospitalization and after hospital discharge (3, 16). Infant well-being continues to be a concern for the mother through the first few months of the infant's life as the baby's health fluctuates widely in this period (22). Approximately 30% of VLBW infants require rehospitalizations during their early months of life (33, 39). Even those infants who have a relatively smooth course require complex caretaking at home that may include apnea monitoring, the administration of multiple medications, and increased contact with health care providers (4). Therefore, mothers of preterm VLBW infants and mothers of healthy term infants may differ in the amount of stress they experience.

Motherhood causes changes in lifestyle which may also influence immune response. Mothers of young infants may skip meals because of time constraints or from a desire to return to prepregnancy weight (19). In nonpostpartal populations dietary imbalances, including inadequate protein (7) and increased fat (30), have been associated with depressed immune function (1, 6, 30). Mothers, once they are no longer pregnant, may increase their amount of cigarette smoking (34), which may be related to decreased immunity by damaging respiratory and vascular tissues or interacting with metabolism and micronutrient intake (9). We have compared immune cell subsets, immune function, anxiety, depression, nutrition, and smoking behaviors in mothers of preterm VLBW infants and mothers of healthy term infants over the first 4 postpartal months to determine if these two groups of postpartal women differ in psychologic and immunologic status.

MATERIALS AND METHODS

Subjects. The study was performed with 60 postpartal women. Thirty women had preterm VLBW infants, and 30 had healthy, normal-weight term infants. Postpartal mothers of term infants were selected so that their age, socioeconomic status, parity, and race were comparable to the mothers of the VLBW infants (Table 1). No mother of a term infant who was ill or septic or who had respiratory difficulties, congenital anomalies, or asphyxia was included in the study. Additionally, women were excluded from the study who had chronic medical and psychiatric problems, any known infection including human immunodeficiency

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TABLE 1. Maternal demographic characteristics[a]

Group	Mean age ± SD (yr)	Race		Annual income ($)		Marital status	
		No. black	No. white	Mean (SD)	Median	No. unmarried	No. married
Mothers of VLBW infants[b]	25 ± 6.7	19	11	20,529 (25,311)	7,278	21	9
Mothers of term infants[c]	25 ± 6.3	19	11	16,280 (19,309)	8,434	20	10
Total	25 ± 6.5	38	22	18,478 (22,530)	7,299	41	19

[a] There were no statistically significant differences for any of the demographic characteristics between the groups.
[b] Mean birth weight, 1,136 ± 274 g.
[c] Mean birth weight, 3,452 ± 578 g.

virus type 1, or hemoglobin levels less than 8 mg/dl following delivery or who had given birth by caesarean section or who were drug or alcohol abusers.

The two groups of mothers were similar in intrapartal and postpartal medication with more than 75% of mothers in both groups receiving epidural anesthesia and with no mothers receiving narcotics for pain when data were collected 2 days following delivery. Although hormonal levels were not measured, all mothers were in the third trimester of pregnancy when they delivered so levels of estrogen and progesterone should have been similar and should have dropped dramatically in both groups within the first 24 h postpartum (40). A minority of women in each group breastfed their infants (8 mothers of VLBW infants and 10 mothers of term infants) and differences in prolactin levels were not measured or compared between the two groups of women.

Measures. Anxiety, depression, and the immune profiles of mothers of VLBW infants and a comparison group of mothers of healthy term infants were examined at delivery and at 1, 2, and 4 months postpartum. Anxiety and depression were measured by the Multiple Affect Adjective Checklist (MAACL) (42). Immune profile was examined with flow cytometry, and on a subset of randomly selected mothers, lymphocyte proliferation assays and NK cell function were assessed. In addition, leukocyte data and general health behaviors were evaluated.

MAACL. The MAACL consists of 132 affect-connoting adjectives, takes approximately 5 min to complete, and provides measures of self-reported moods. Scoring is bipolar (42). A revised scoring system has been developed but it is the original scoring that we have used in all data analysis (25). Across the anxiety and depression scales the median internal reliability estimate over eight samples was reported as 0.85 (range, 0.69 to 0.95). Concurrent validity is supported by the correlation in anxiety scores between the MAACL and Spielberger's State-Trait Anxiety Inventory ($r = 0.66$) (17, 37). In the present study the internal consistency reliability was 0.82 for anxiety and 0.81 for depression.

Immunologic studies. Peripheral venous blood was obtained from mothers, and complete blood counts and differential analyses were performed with a Coulter STKS (an automated cell counting and flow cytometric scatter analysis system) (Coulter Electronics, Hialeah, Fla.). Additionally, cell phenotypes were determined including CD3 (pan T cell), CD4 (helper T cell), CD8 (suppressor, cytotoxic T cell), CD11b (iC3B receptor), $CD4^+/CD29^+$ (a CD4-positive lymphocyte inducer helper cell), $CD4^+/CD45RA^+$ (a CD4-positive lymphocyte inducer suppressor cell), and HLA Class II Ia on lymphocytes (polymorphic Class II antigen). The levels of B-cell markers (CD20), monocyte markers (including CD11b, CD14, and Ia), $CD3^-/CD16^+$ (NK-Fc receptor), $CD3^-/CD56^+$ (NK cell marker), and $CD3^-/CD57^+$ (NK cell marker) were also determined. It is important to study multiple markers of NK cell populations, e.g., $CD3^-/CD16^+$, $CD3^-/CD56^+$, and $CD3^-/CD57^+$, which are correlates in depressive disorders (13).

Cell phenotypes in circulating peripheral blood were measured with fluorescence-activated flow cytometry. Briefly, 0.02 ml of the appropriately diluted fluorochrome-conjugated monoclonal antibody was added to 0.1 ml of whole blood. After incubation for 30 min at 4°C, 2 ml of lysing buffer, maintained at 37°C under 5% CO_2 (8.26 g of ammonium chloride/liter, 1.00 g of potassium bicarbonate/liter, and 0.037 g of EDTA/liter [pH 7.3]), was added to each sample, and samples were incubated for 1.5 min to lyse erythrocytes. This was followed by a final wash with phosphate-buffered saline (PBS). The samples were then fixed by the addition of 0.5 ml of 1% paraformaldehyde prepared in PBS (pH 7.3). Samples were stored at 4°C in the dark until being evaluated by flow cytometry. Whole-blood specimens routinely stood for 6 to 24 h at room temperature prior to staining, which is well within the time frame necessary to avoid unacceptable results attributable to elevated nonviability.

A Coulter Epics Elite Flow cytometer operated at 488 nm and 300 mW of output was used for all immunophenotypic studies. Lymphocytes and monocytes were gated based on their physical scatter characteristics and fluorescence by employing the criteria that the lymphocyte cluster must be greater than 95% positive for CD45 and less than 2% positive for CD14, while the monocyte cluster must be greater than 80% positive for CD14. A higher gating criterion for monocytes (e.g., 90% positive for CD14) was originally used. However, monocyte data would not have been interpretable for many of the mothers given this criterion. The 80% cutoff allowed for relative assurance that all monocytes were being counted. In other studies monocyte clusters with lower CD14 expression have also been demonstrated (24).

Lymphocyte responses in vitro to phytohemagglutinin (PHA), concanavalin A (ConA), and proliferative pokeweed mitogen (PWM) were assessed for a subsample of five mothers of VLBW infants and their five matched control mothers (15). We also assessed NK function as determined by chromium release from the target cell K562. The subsample of mothers of VLBW infants was randomly selected, by the sealed envelope technique, from all mothers of preterm VLBW infants who had idiopathic preterm deliveries, and functional assays were also performed for five matched controls.

Lymphocyte mitogen stimulation assay. The assay procedure involves the purification of peripheral blood mononuclear cells by Ficoll-Hypaque density gradient centrifugation. The mononuclear cells were then placed in culture in triplicate (10^5 cells/200 μl of RPMI medium) followed by stimulation with optimal doses of PHA, ConA, and PWM. After 72 h of incubation, the cell cultures were each pulsed with 0.5 μCi of [^{3}H]TdR (10 Ci/mmol) for 6 h and harvested. The uptake of radioisotope for unstimulated and mitogen-stimulated cultures was then measured by liquid scintillation spectrometry and calculated as net radioactivity (mitogen-stimulated counts per minute [cpm] − unstimulated cpm).

NK cell functional activity. Peripheral blood-derived mononuclear cells were used as effector cells and were prepared from 25 ml of heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. NK cytotoxicity was evaluated by the release of ^{51}Cr from isotopically labeled target cells (K562). Fresh or rapidly thawed cryopreserved target cells were washed and resuspended in 0.2 ml of Hanks' balanced salt solution and labelled with ^{51}Cr by the addition of 50 μCi of $Na_2{}^{51}CrO_4$ (2.0 Ci/mmol) for each 2×10^6 cells. After a 1-h incubation at 37°C in 5% CO_2 with gentle agitation, the cells were washed four times and resuspended in RPMI 1640 medium containing 10% fetal bovine serum (14).

Labeled target cells in 0.1-ml aliquots were added to round-bottomed microtiter plates, which was followed by the addition of serially diluted effector cells to achieve appropriate effector cell to target cell ratios. The plates were centrifuged and incubated for 4 h (K562 cell targets). One-tenth of a milliliter of supernatant fluid was removed at appropriate time points (in triplicate) for the evaluation of the ^{51}Cr released from lysed cells. Total release activity was determined by ^{51}Cr release from culture wells treated with 1% Triton X-100, which routinely releases more than 80% of the isotype from labeled target cells. Spontaneous release was obtained from target cells incubated with medium alone. K562 targets release less than 5% of label.

The percentage of specific ^{51}Cr released was calculated as (cpm experimental − cpm spontaneous)/(cpm total − cpm spontaneous) × 100.

Health behaviors. Cotinine was measured in maternal serum from all mothers at every data point by gas chromatography. Nutritional intake was measured by 24-h diet recall that was documented by the mother and then discussed with the mother for accuracy by the research assistant. Diet recalls were done on weekdays as well as on weekends, and food models were used with mothers to help them more accurately document portion sizes.

Statistical methods. Data were analyzed with the BMDP statistical software package. Baseline (at delivery) demographic variables and presence of cotinine and caffeine were compared between the two groups (VLBW and term) by using Fisher's exact test. Anxiety, depression, leukocyte levels, immunologic marker values, and nutrition variables were compared by using a t test, after a log transformation to reduce skewness when necessary. The decision to transform was based on histogram results. In the cases where transformed values were used, the geometric mean on the original scale is given as well as the median absolute deviation estimator since the standard deviation on the original scale is not an appropriate measure of spread. Repeated measures analysis of variance models were fit to the mental state variables, leukocyte data, immunologic markers, and health behaviors to examine whether the two groups changed over time and whether the pattern of change over time was similar or different between the two groups, above and beyond any baseline differences. The models used the actual time since delivery in the fit, and both linear and quadratic time effects were fit. The choice of whether to use the quadratic fit or only the linear term depended both on the significance of the quadratic coefficient and the size of the residuals. Lymphocyte responses in vitro were analyzed similarly.

TABLE 2. Mental state and immunologic parameters at delivery

Characteristic	Mean score[b] or % positive ± SD			P
	Mothers of VLBW infants	Mothers of term infants	Total	
Anxiety	7.17 ± 3.75	4.57 ± 2.93	5.87 ± 3.59	0.004
Depression	12.67 ± 6.21	10.50 ± 4.30	11.58 ± 5.41	0.122
T-cell marker				
CD8	16.57 ± 10.33	21.49 ± 8.26	19.03 ± 9.59	0.050
CD4	46.57 ± 9.82	46.09 ± 12.43	46.33 ± 11.09	0.870
CD29	38.56 ± 16.04	43.21 ± 15.24	40.88 ± 15.68	0.262
CD11b (lymphocyte)	5.26 ± 4.36	8.11 ± 6.51	6.74 ± 5.71	0.055
Ia (lymphocyte)	13.00 ± 6.40	15.69 ± 7.09	14.35 ± 6.83	0.128
CD3	79.98 ± 7.50	82.42 ± 6.87	81.20 ± 7.24	0.193
CD45RA	36.56 ± 18.53	43.01 ± 16.77	39.79 ± 17.81	0.170
Monocyte marker				
CD11b	77.45 ± 31.61	92.62 ± 10.47	85.73 ± 23.69	0.029
CD14	81.06 ± 14.39	91.74 ± 7.75	86.68 ± 12.50	0.001
Ia	76.95 ± 29.61	88.96 ± 14.16	83.27 ± 23.40	0.063
B-cell marker[a]				
CD20	5.00 (2.75–9.88)	7.61 (5.65–12.10)	6.17 (4.30–10.40)	0.036
NK cell marker[a]				
$CD3^-/CD16^+$	2.64 (1.50–5.05)	4.22 (2.65–7.45)	3.35 (2.10–6.20)	0.042
$CD3^-/CD56^+$	3.74 (2.00–7.25)	6.11 (3.50–11.20)	4.81 (2.90–9.30)	0.025
$CD3^-/CD57^+$	1.77 (0.95–3.10)	2.08 (1.30–4.15)	1.92 (1.15–3.60)	0.469

[a] Values for B-cell and NK cell markers are geometric means (interquartile ranges are shown in parentheses).
[b] For anxiety and depression.

RESULTS

(i) Comparisons at delivery. There were no statistically significant differences between the mothers of the VLBW infants and the mothers of the term infants for any of the demographic variables (Table 1). All leukocyte parameters were compared by using a *t* test after a natural log transformation to reduce skewness. There were no significant differences between the two groups.

A summary of the mental state variables and the immunologic parameters at delivery is given in Table 2. The mothers of VLBW infants had higher anxiety ($P = 0.004$) but not higher depression ($P = 0.12$) immediately following delivery compared with the mothers of term infants. Mothers of VLBW infants had lower levels of CD8 at delivery (16.6%) than did mothers of term infants (21.5%) ($P = 0.05$). The percentages of CD11b on lymphocytes was 5.3 and 8.1% for mothers of VLBW and term infants, respectively ($P = 0.055$). There were also differences in CD20 levels, a B-cell subset, which were lower in mothers of VLBW preterm infants than in mothers of term infants ($P = 0.036$) (geometric means, 5.0 and 7.6, respectively).

The three monocyte markers (CD14, Ia, and CD11b) were either significantly or borderline higher in the mothers of term infants than in mothers of VLBW infants. The percentages of NK cells was significantly higher for both $CD3^-/CD16^+$ and $CD3^-/CD56^+$ in mothers of term infants ($P = 0.04$ and $P = 0.03$, respectively) but were not different for $CD3^-/CD57^+$.

(ii) Longitudinal comparison of anxiety, depression, and immunological markers. Repeated measures models were fit to all parameters (Table 3). The *P* value represents whether the changes over time were similar or different between the mothers of VLBW infants and the mothers of term infants (interaction effect) above and beyond differences at delivery. Group differences (in anxiety) and time differences ($CD4^+/CD29^+$, CD11b lymphocytes, and total activated T cells) are discussed below but are not shown in Table 3.

The results for mothers of VLBW infants showed statistically significant differences in anxiety compared with the results for mothers of term infants ($P = 0.0096$). Anxiety continued to be higher in mothers of VLBW infants than in mothers of term infants at every data point and did not significantly change over time until 4 months postpartum in either group ($P = 0.63$). There were no significant differences in depression between the two groups, and similar to anxiety, levels stayed the same across time.

There were no significant differences in total leukocyte counts between mothers of VLBW infants and mothers of term infants ($P = 0.70$). Both groups of mothers started out at essentially the same point (10.4×10^3 cells for the VLBW group versus 11.0×10^3 cells for the term group), which dropped quickly over time ($P < 0.00005$) so that by the first postpartal month the leukocyte counts were 6.2 and 6.1, respectively, for mothers of VLBW and of term infants. Both groups had the same pattern of change over time ($P = 0.16$) with no further changes occurring after the first postpartal month.

Both the mothers of VLBW infants and the mothers of term infants started out at essentially the same percentage in lymphocytes, but the mothers of VLBW infants ended at a slightly lower percentage than the mothers of term infants, with the groups having changed at a different rate across time ($P = 0.0002$). Neutrophil levels also were similar at delivery but decreased at a slower rate in mothers of VLBW infants than in mothers of term infants ($P = 0.006$). Eosinophils increased more in the mothers of term infants than in mothers of VLBW infants ($P = 0.001$).

For all the T-cell markers, the quadratic model provided a better fit than the linear model. In most parameters this reflected a phenomenon of decreased values at delivery and an increase by 1 month postpartum. This increase was sustained, in most cases throughout the 4-month period, although in some cases, there was a decrease by the 4-month measure-

TABLE 3. Differences in anxiety, depression, and immunologic markers

Characteristic	Mean and dispersion								P^a
	Mothers of VLBW infants				Mothers of term infants				
	Delivery	1 mo	2 mo	4 mo	Delivery	1 mo	2 mo	4 mo	
Anxiety	7.2 ± 3.8	6.0 ± 4.2	6.2 ± 4.2	6.8 ± 4.2	4.6 ± 2.9	4.8 ± 2.9	5.7 ± 3.6	5.2 ± 3.4	0.63
Depression	12.7 ± 6.2	11.0 ± 7.2	12.0 ± 7.7	12.9 ± 7.7	10.5 ± 4.3	10.7 ± 5.2	11.5 ± 5.8	10.0 ± 6.6	0.29
Leukocyte[b]	10.4 ± 1.3	6.2 ± 1.0	6.5 ± 1.2	6.4 ± 1.0	11.0 ± 1.8	6.1 ± 1.3	6.0 ± 0.9	5.9 ± 0.6	0.16
Lymphocyte[c]	18.0 (6.4)	38.3 (5.8)	38.9 (7.18)	33.1 (5.7)	16.6 (5.1)	35.5 (8.5)	35.2 (4.5)	38.1 (6.5)	0.0002*
Eosinophil[c]	1.8 (0.5)	2.6 (0.9)	2.0 (0.7)	2.0 (0.6)	1.3 (0.7)	3.0 (0.9)	2.9 (1.3)	3.2 (1.2)	0.001*
Neutrophil[c]	70.8 (7.8)	49.4 (7.3)	47.0 (7.3)	54.6 (7.53)	72.2 (5.7)	48.4 (9.8)	50.4 (7.2)	47.9 (6.1)	0.006*
Monocyte	4.6 ± 2.8	6.8 ± 1.3	6.8 ± 2.2	6.5 ± 1.0	4.7 ± 2.5	6.6 ± 1.6	6.4 ± 2.1	5.8 ± 2.5	0.59
Basophil	0.9 ± 0.5	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.5	0.6 ± 0.3	0.7 ± 0.5	0.7 ± 0.4	0.6 ± 0.5	0.72
CD3	80.0 ± 7.5	81.1 ± 7.3	80.3 ± 6.1	81.4 ± 6.5	82.4 ± 6.9	82.6 ± 5.5	82.2 ± 7.2	82.2 ± 6.0	0.65
CD4	46.6 ± 9.8	44.6 ± 8.2	43.7 ± 10.0	45.9 ± 9.5	46.1 ± 12.4	47.2 ± 9.2	48.2 ± 7.6	48.2 ± 7.6	0.22
CD8[c]	16.0 (10.3)	22.7 (10.1)	23.9 (11.1)	22.1 (8.4)	21.5 (8.3)	22.5 (10.1)	21.9 (8.1)	20.5 (8.4)	0.005*
Total active T cell	10.7 ± 8.0	12.5 ± 8.5	15.1 ± 8.7	14.0 ± 8.6	9.6 ± 5.1	12.2 ± 6.9	11.5 ± 4.9	10.9 ± 5.5	0.87
$CD4^+/CD29^{+d}$	38.6 ± 16.0	47.0 ± 14.9	48.2 ± 17.6	53.4 ± 12.7	43.2 ± 15.2	49.9 ± 11.6	53.1 ± 11.7	51.4 ± 10.0	0.60
$CD4^+/CD45RA^{+d}$	36.6 ± 18.5	40.7 ± 15.5	36.2 ± 16.0	39.4 ± 15.9	43.0 ± 16.8	43.1 ± 13.8	43.8 ± 13.3	39.7 ± 13.3	0.62
CD11b (lymphocyte)	5.3 ± 4.4	9.9 ± 7.5	11.1 ± 9.0	11.1 ± 7.8	8.1 ± 6.5	9.5 ± 6.7	9.9 ± 5.8	8.9 ± 6.4	0.40
Ia (lymphocyte)	13.0 ± 6.4	11.7 ± 4.4	15.1 ± 6.2	13.3 ± 4.2	15.7 ± 7.1	13.3 ± 5.0	14.7 ± 5.2	14.2 ± 6.9	0.14
CD20[c]	5.0 (3.4)	5.6 (1.4)	7.3 (3.7)	8.4 (1.6)	7.6 (2.6)	6.1 (2.2)	8.2 (2.8)	7.5 (3.1)	0.0045*
$CD3^-/CD16^+$	2.6 ± 1.7	5.4 ± 2.3	6.2 ± 2.1	5.4 ± 2.4	4.2 ± 2.0	5.3 ± 1.7	4.9 ± 2.0	5.5 ± 1.9	0.61
$CD3^-/CD56^{+c}$	3.7 (2.4)	6.2 (3.4)	5.9 (2.9)	7.2 (3.7)	6.1 (3.1)	6.0 (1.7)	6.2 (3.6)	6.5 (2.2)	0.013*
$CD3^-/CD57^+$	1.8 ± 1.1	2.9 ± 1.3	3.5 ± 1.7	2.8 ± 1.2	2.1 ± 1.1	2.8 ± 1.5	2.8 ± 1.2	2.8 ± 1.6	0.74
CD14[c]	81.1 (14.4)	84.0 (12.9)	86.1 (11.0)	89.3 (11.5)	91.7 (7.7)	88.7 (10.6)	91.5 (12.1)	89.3 (9.9)	0.0007*
CD11b (monocyte)	77.4 ± 31.6	87.5 ± 15.9	88.3 ± 19.3	88.5 ± 16.5	92.6 ± 10.5	86.1 ± 17.3	90.1 ± 11.6	89.4 ± 16.4	0.60
Ia (monocyte)[c]	77.0 (29.6)	85.3 (18.2)	90.1 (20.0)	95.3 (8.6)	89.0 (14.2)	92.7 (15.2)	94.6 (16.9)	95.1 (8.18)	0.025*

[a] P values indicate significance of changes over time between groups determined by repeated measures analysis of variance. *, <0.05.
[b] Values are absolute cell counts ($10^3/mm^3$).
[c] Values are geometric means, and median absolute deviations are in parentheses.
[d] Values are percentages of total $CD4^+$ cells which coexpress CD29 or CD45RA.

ment. Among the T-cell markers, CD8 levels showed differences between groups over time, with CD8 percentages in mothers of VLBW infants increasing to levels comparable to those of mothers of term infants by 1 month and to higher levels at 2 and 4 months ($P = 0.005$) (Fig. 1).

The percentages of $CD4^+/CD29^+$ were lower at delivery than they were later in the postpartal period in both groups (average of 41% at delivery that increased by 4 months postpartum to 52%; $P < 0.0027$). The percentages of total activated T cells ($CD3^+/CD57^+$) also increased over time (average of 10.15 at delivery versus 12.45% at 4 months; $P = 0.0001$). This pattern of comparably lower rates at baseline for mothers of VLBW infants and mothers of term infants rising to comparably higher levels at 4 months postpartum also held true for CD11b receptor-bearing lymphocytes (5.3% in mothers of VLBW infants and 8.1% in mothers of term infants; $P = 0.0025$). CD3, CD4, $CD4^+/CD45RA^+$, and Ia on lymphocytes showed no differences, either at delivery or at later datum points, between the two groups.

A linear model fit the monocyte marker data well. At delivery the percentage of CD14 for the mothers of VLBW infants was 81%, and it was 92% for the mothers of term infants ($P = 0.001$). Within the first 4 months, mothers of VLBW infants had lower percentages of CD14 at every time point. However, mothers of VLBW infants averaged an increase of 2.2% in CD14 per month, while mothers of healthy term infants had an average decrease of 0.7%, so that at 4 months postpartum the levels for both groups were approximately 89% ($P = 0.0007$). This pattern of a higher baseline with higher levels in the mothers of term infants until 4 months postpartum was also true for Ia expression on monocytes. The mothers of VLBW infants started at 77%, while the mothers of term infants started at 89%, and both groups reached 95% by 4 months, with a different rate of change ($P = 0.025$). However, there was no difference between the two groups in the level of CD11b monocytes.

The B-cell marker CD20 had a skewed distribution and was analyzed on the natural logarithm scale. There was a difference at delivery of approximately 2.6% ($P = 0.036$), with mothers of VLBW infants having lower values than the mothers of term infants. The mothers of VLBW infants showed a quicker rate of increase over the 4-month period than the mothers of term infants ($P = 0.0045$). By the end of 4 months, the geometric means were approximately 8.4% in the mothers of VLBW infants and 7.5% in the mothers of term infants. The NK cell marker data were skewed and were analyzed on the natural logarithm scale. The $CD3^-/CD56^+$ patterns were different across time. While percentage of $CD3^-/CD56^+$ for the mothers of VLBW infants showed a steady increase from a geometric mean of 3.7 to 7.2%, the percentage for this marker for the mothers of term infants remained stable at approximately 6.2% for the 4 postpartal months ($P = 0.013$). The two other NK markers ($CD3^-/CD16^+$ and $CD3^-/CD57^+$) showed no differences during the first 4 months. NK cell assays did not demonstrate differences between the two groups over time.

(iii) Lymphocyte proliferation and NK cell assays. Lymphocyte responses in vitro and NK functions were assessed in five randomly chosen mothers in each group, at all time points. In most cases the lymphocyte responses were lower in the VLBW mothers than in the term mothers ($P < 0.005$ for most comparisons) (Table 4).

In NK functional activity, there were no differences between mothers of term infants and mothers of VLBW infants at delivery. The mean chromium release at dilutions of both 50 and 25 showed differences across time ($P < 0.00005$ and $P = 0.03$, respectively). The NK function decreased faster in the

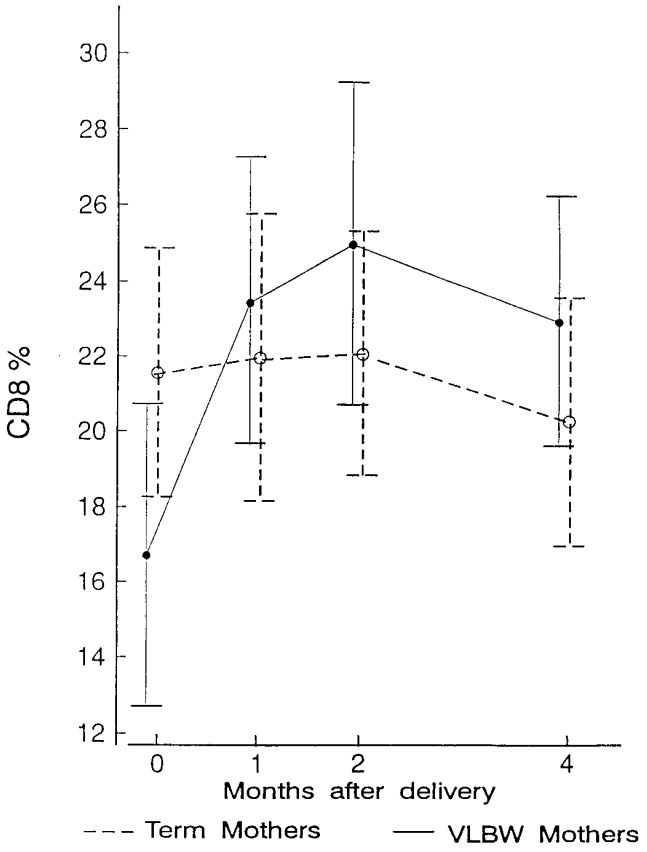

FIG. 1. CD8 percentages at delivery and at 1, 2, and 4 months after delivery for mothers of VLBW infants and mothers of term infants. Values are means, and 95% confidence intervals are shown (error bars).

VLBW mothers than in the term mothers. This result was also observed at one more dilution (12.5) ($P = 0.054$). However, because of the small sample size, these results should be interpreted cautiously and an assessment of magnitude of effect is not reliable at this point.

(iv) Postpartal health behaviors: smoking and nutrition. There were no differences between the two groups of mothers attributable to smoking behavior. When smoking was examined to determine if it had an effect on the immune profile (by using cotinine level as a covariate in the repeated measures analysis), only one of the immune parameters from Table 3 showed any significance and this was marginal, especially in light of the large number of parameters being examined. There were no statistically significant differences between mothers of term infants and mothers of VLBW infants in protein, calorie, or fat intake.

Nutritional status was related to immune function in the postpartal women. Although there were no differences between mothers of VLBW infants and mothers of term infants in total calories, the percentage of calories ingested from protein, or the percentage of calories that were obtained from fat, at any of the data points, there was a significant difference between women with high-fat and normal fat diets in $CD3^-/CD57^+$ percentages (an NK subset) ($P = 0.01$) at delivery. At 2 months there were significant differences between mothers with high-fat and normal fat diets in CD14 (monocyte) ($P = 0.02$) and $CD4^+/CD45RA^+$ percentages (inducer-suppressor T-cell subset) ($P = 0.02$), with women with high-fat diets having lower percentages of cell subsets at each data point. Per-

TABLE 4. Differences in lymphocyte proliferation assays

Mitogen (μg/ml)	Mean lymphocyte proliferation (cpm) ± SEM					
	Mothers of VLBW infants			Mothers of term infants		
	Delivery	1 mo	2 mo	4 mo	Delivery	1 mo	2 mo	4 mo
PHA								
8	59,732.80 ± 19,246.39	62,466.67 ± 15,964.60	66,126.6 ± 23,644.92	59,271.60 ± 13,488.47	92,651.60 ± 13,481.33	118,635.50 ± 22,032.00	112,170.25 ± 19,652.55	93,663.00 ± 12,242.86
4	39,443.80 ± 15,357.32	45,548.50 ± 7,834.59	50,552.00 ± 17,304.58	42,042.80 ± 13,100.21	74,359.20 ± 16,455.00	75,622.25 ± 23,277.07	75,471.00 ± 13,790.05	41,245.20 ± 5,448.04
PWM								
12.5	32,711.80 ± 5,039.27	23,535.50 ± 1,929.51	22,845.40 ± 8,630.16	20,030.40 ± 2,632.56	27,451.00 ± 5,912.44	33,016.5 ± 2,983.12	28,340.75 ± 5,740.54	21,299.40 ± 4,198.38
6	49,049.20 ± 10,016.72	17,450.25 ± 5,439.23	24,805.60 ± 10,235.88	20,477.80 ± 2,416.98	27,023.80 ± 5,922.35	33,976.75 ± 1,808.73	31,664.50 ± 6,807.44	24,126.40 ± 4,889.64
ConA								
2.5	24,090.20 ± 6,604.11	12,926.50 ± 3,155.25	10,580.80 ± 5,195.31	10,719.80 ± 3,987.97	15,773.00 ± 3,885.36	29,106.75 ± 2,467.80	41,166.75 ± 12,161.90	20,845.80 ± 5,215.61
	12,580.40 ± 3,045.64	8,130.00 ± 2,579.04	6,958.60 ± 6,739.83	18,271.40 ± 15,147.87	5,563.60 ± 1,774.12	13,365.75 ± 2,807.96	21,255.50 ± 7,828.42	9,126.80 ± 2,319.10

haps dietary influences on NK cell functioning overrode any difference that might have been observed between mothers of term infants and mothers of VLBW infants in NK cell response to stress.

Women with low-protein, low-calorie diets had significantly lower percentages of $CD3^-/CD57^+$ (NK cell subset) at delivery ($P = 0.007$) as well as significantly lower percentages of $CD4^+/CD29^+$ (inducer-helper T-cell subset) ($P = 0.03$). At 2 months postpartum women with low-calorie and low-protein diets had significantly lower percentages of CD11b lymphocytes ($P = 0.01$), and at 4 months postpartum, women with low-protein, low-calorie diets had significantly lower levels of total activated T cells ($P = 0.004$) and Ia on lymphocytes ($P = 0.01$).

DISCUSSION

The leukocytosis of pregnancy is due primarily to a granulocytosis (8). This granulocytosis was comparable in both the mothers of VLBW infants and mothers of term infants and returned to baseline by the first postpartal month. The changes in the level of total leukocytes may be related to cortisol levels, catecholamine, or other endocrine factors or leukotrenes in pregnancy. While there have been several studies of lymphocyte subsets and mitogenic responses during pregnancy, heretofore there have been no investigations of the role of a major stressor, the delivery of a VLBW infant, on immunologic markers in these women. Mothers of VLBW infants experienced higher anxiety over time which did not resolve until 4 months postpartum. Concomitantly, our data show some decrease in the percentages of lymphocytes and eosinophils. These decreases may be related to stress and increased corticosteroid levels or, perhaps, to estrogen levels.

We also observed important differences between mothers of VLBW infants and mothers of term infants in the expression of Ia on monocytes, which were reduced at delivery in the mothers of VLBW infants and continued to be reduced at 1 month with recovery at 2 and 4 months. The expression of CD14 on cells gated as monocytes was reduced for the mothers of VLBW infants at delivery, 1, and 2 months and recovered at 4 months. In contrast, CD11b expression on monocytes was not significantly different between mothers of VLBW infants and term mothers. These differences indicate that the changes in the cell surface display of molecules on monocytes may be related to monocyte activation, namely Ia and CD14. CD14 is a major receptor for lipopolysaccharide on monocytes (41). There are several possible interpretations of these observations. One is the possibility that altered expression of CD14 and Ia in the absence of changes in CD11b levels (the complement receptor) reflect a response of monocytes to stress, namely caring for a VLBW preterm infant. Another possibility is that there is a shift in the composition of circulating monocyte populations and that these differ between mothers of term infants and mothers of VLBW infants. A recent study has shown diminished expression of CD14 on neonatal monocytes and neutrophils (35). Furthermore, the expression of these receptors may be altered by steroid, endocrine, or other serum-derived factors in mothers of VLBW infants. For example, although most women did not breastfeed, differences in prolactin levels may have contributed to differences between the groups.

Some postpartal changes in subsets of T cells which have been reported by others, such as a decline in CD4 accompanied by an increase in CD8 and a postpartal increase in $CD4^+/CD45RA^+$ (38), were not found in this study. CD4 levels and $CD4^+/CD45RA^+$ levels remained stable in both groups of mothers. Inducer-suppressor cells ($CD4^+/CD45RA^+$) (32) may have an important role in postpartal immune dysfunction such as postpartal thyroiditis (38). $CD4^+/CD45RA^+$ cells are also elevated in patients with certain primary immune deficiencies such as chronic mucocutaneous candidiasis (29).

Although CD8 levels did rise in mothers of VLBW infants, it actually decreased in mothers of term infants. The levels of CD8 at delivery (mothers of VLBW infants, 16%; mothers of term infants, 21%) and at 4 months postpartum (22% in both groups of mothers) were relatively low compared to the work of Stagnaro-Green and colleagues (38), who reported CD8 levels of 30.5 and 31.3% at 3 and 6 months postpartum for a group of normal postpartum women. The basis for these differences may be related to the monoclonal antibody used, the flow cytometry gating techniques, or demographic features of the population studied.

The mothers in our study were primarily from a lower socioeconomic background, and health behaviors such as poor nutrition and lack of exercise may have had an effect on immune profiles in both the mothers of VLBW infants and the mothers of term infants, which make comparisons difficult between this sample of mothers and more advantaged postpartal women. Differences between the mothers of VLBW infants and the mothers of term infants within the study were controlled methodologically by matching mothers of VLBW infants and mothers of term infants on parity, age, socioeconomic level, and race, and no statistical differences were found between groups when lifestyle variables were controlled (see Table 1). Therefore, we are confident that differences between the two groups of mothers in immune profiles cannot be attributed to differences in lifestyle variables. Without a nonpostpartal control group of women matched for age, race, and socioeconomic status it is difficult to know if these nutritional inadequacies are unique to the postpartal period or if they are related to other factors such as socioeconomic status. For both mothers of term infants and mothers of VLBW infants who are poor, inadequate nutrition may decrease the number of peripheral blood lymphocytes, monocytes, and NK cells present.

The poorer lymphocyte proliferative response to PHA, ConA, and PWM demonstrated by mothers of VLBW infants compared to mothers of term infants is very likely to be indicative of a stress response (23). At each of two dilutions for three different mitogens mothers of VLBW infants continually demonstrated less proliferative responses. This supports earlier findings of decreased lymphocyte responses in stressed individuals (26).

In studies of men with human immunodeficiency virus, severe stress was associated with reductions in NK cell populations and in CD8 counts, T cells which are related to cytotoxic effector T cells (12, 13). The results of our studies are consistent with the stress-related alterations in these subsets but further analysis is necessary, especially analysis that controls for endocrine changes.

The mothers of VLBW infants in this study experienced increased anxiety, decreased lymphocyte proliferation, and decreased percentages of some immunologic cell subsets during the first 4 postpartal months compared to mothers of healthy term infants. The postpartal period is a time when the immunosuppression associated with pregnancy is still resolving, and mothers of preterm, VLBW infants appear to differ from mothers of healthy term infants in immune status in the early postpartal period.

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