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Lymphocyte, Monocyte, and Natural Killer Cell Reference Ranges in Postpartal Women

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Normative values for immune-cell subsets in postpartal women, who are recovering from the relative immunosuppression of pregnancy, have not been established. Considerable differences in normative values for subsets of immune cells have been demonstrated based on sociodemographic factors, such as age and race. In order to make accurate clinical decisions about postpartal women, comparisons with normal reference ranges are necessary. Therefore, flow cytometric data for 51 healthy women at 4 months postpartum are presented and changes over the first 4 postpartal months are documented. The levels of some lymphocyte cell subsets, such as $CD4^+/CD45RA^+$ and Ia on lymphocytes, remained stable over time. The levels of other lymphocyte cell subsets, such as $CD4^+/CD29^+$, increased over the first 4 postpartal months, while those of other cell subsets, such as CD8 and CD11b, increased between delivery and 2 months postpartum and then dropped again by the fourth postpartal month. The levels of two natural killer cell subsets ($CD3^-/CD16^+$ and $CD3^-/CD57^+$) rose from delivery until 1 month postpartum and then plateaued. Comparisons were made with reference ranges of nonpostpartal groups provided in the literature and in a study of healthy women being conducted in the same laboratory, and postpartal women were found to have lower values of CD8, $CD3^-/CD16^+$, $CD4^+/CD45RA^+$, CD20, and CD11b than those reported in the literature.

The duration of the immunosuppression of pregnancy into the postpartal period has not been fully delineated. Although not all investigators have documented differences between T and B lymphocytes and natural killer (NK) cells from the third trimester of pregnancy through the first 5 postpartum months (3, 7), most researchers have found that T-cell subset numbers decrease during pregnancy and return to normal in the postpartal period. However, there is disagreement about the time frame within which return to nonpregnant immune baseline status occurs.

Some studies have found that peripheral blood or circulating lymphocytes return to nonpregnant rates within the first few postpartal weeks (19, 23). In contrast, other studies have documented that postpartal return to normal takes between 3 and 9 months (2, 8, 15, 22). In some studies, lymphocyte cell numbers and immunoglobulin levels were elevated in the first 4 postpartal months, as evidenced by increases in the levels of immunoglobulin G and in the numbers of CD3 and CD56 lymphocytes over normal nonpregnant levels (13). This increased immunologic activity, if indeed it is a reproducible finding, might be a compensatory mechanism as the body adjusts from pregnancy-induced immunosuppression. Alternatively, increased immune activity in the postpartal period may be a natural mechanism to decrease the likelihood of maternal morbidity from postpartal infection (10, 13).

A portion of the variability in findings regarding postpartal immune status may be due to methodologic issues. Erythrocyte rosetting, now outmoded, is sensitive to variations in technique and thus has been replaced by flow cytometry. Likewise, some older studies used immunofluorescence microscopy rather than flow cytometry to enumerate lymphocytes, and accuracy in counting large numbers of cells by microscopic quantification is difficult to achieve. Some mononuclear cell subsets, such as monocytes, are difficult to enumerate. Flow cytometry is much more accurate in counting monocytes than are automated hematology analyzers; however, sample preparation techniques have an effect on monocyte count, although the antibody clone investigated does not seem to alter monocyte count (11).

Even when flow cytometry is used, the lack of published reference values for a relatively young, female, postpartal group makes it difficult to compare findings between laboratories. Consensus is only now being reached on how values for flow cytometry should be standardized for certain cell subsets, and consensus has not yet been reached for many other cell subsets (16). Additionally, it is difficult to compare immune results because of the wide variations which exist in how results are reported, including various measures of central tendency (mean or median) as well as numerous measures of variability (confidence intervals, interquartile ranges, standard errors of the mean, and standard deviations) (12).

Considerable differences in normative values for subsets of lymphocytes and monocytes have been demonstrated based on sociodemographic factors. For example, as individuals age, there are normal decreases in the number of peripheral blood lymphocytes (20). Additionally, men have higher values for cell subsets than do women (16). There are also racial differences for some cell subsets. For example, African-Americans have higher levels of CD3 and $HLA\text{-}DR^+$ cells than do Caucasians (20, 21). Therefore, it is important to have reference ranges obtained from healthy postpartal women to use in making clinical decisions about postpartal women.

Exacerbation of immune-mediated disease in the postpartum occurs in a number of conditions, such as ulcerative colitis, rheumatoid arthritis, and systemic lupus erythematosus (14,

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TABLE 1. Sources of monoclonal antibodies

Monoclonal antibody[a]	Cellular distribution of antigen function	Vendor
CD14 FITC	Monocytes, granulocytes, macrophages	AMAC
CD45 FITC	Leukocyte common antigen	AMAC
CD16 FITC	NK cells, granulocytes, macrophages	AMAC
CD3 PE	T cells, thymocytes	AMAC
CD57 FITC	NK cells, T-cell subsets	Becton Dickinson
CD56 PE	NK cells	AMAC
CD3 FITC	T cells, thymocytes	AMAC
CD4 FITC/CD29 PE	CD4—helper-inducer T-cell subset, monocytes, thymocytes; CD29—subset of $CD4^+$ T cells	Coulter
CD4 FITC/CD45RA PE	CD4—helper-inducer T-cell subset, monocytes, thymocytes; CD45RA—B cells, monocytes, $CD4^+$ T-cell subset	Coulter
CD8 FITC	Cytotoxic/suppressor T-cell subset, ND cells, thymocytes	AMAC
CD20 FITC	B-cell restricted	AMAC
HLA-DR FITC	Cells expressing polymorphic class II antigens	AMAC
CD11b FITC	Granulocytes, monocytes, NK cells	AMAC

[a] FITC, fluorescein isothiocyanate; PE, phycoerythrin.

25). Additionally, one of the most common postpartum diseases is postpartum thyroid dysfunction. In treating these and other immune-mediated diseases, reference values for immune-cell subsets in healthy women might prove of immense value. For example, recent studies have found that the levels of $CD45RA^+$ T cells are significantly elevated in women who have postpartal thyroid disease compared to those in healthy postpartal women (22).

MATERIALS AND METHODS

Subjects. Fifty-one postpartal women comprised the sample of this study, which was part of a larger study comparing the immune responses in mothers of very low birthweight (VLBW; ≤1,500 g) and term infants (NR02615). Healthy mothers of term infants were entered into the larger study to match the sociodemographic characteristics of mothers of VLBW infants. Of 51 mothers of healthy term infants, 33 were African-American (65%) and 18 were Caucasian (35%). Most mothers were unmarried (67%) and were multiparous (53%). The mean maternal age was 25 years (standard deviation = 6). As part of the larger study, dietary habits were measured, and this group of women had diets that were high in fat (75% of women had >30% of daily calories from fat).

Peripheral blood specimens from nonpostpartal controls for a study of human papillomavirus were examined in our laboratory during the same period that the specimens for postpartal subjects were being evaluated (17). These controls were 112 healthy women with a mean age of 27, of which 74% were African-American or West Indian, 13% were Caucasian, 7% were Hispanic, and 6% were other. The control data for healthy nonpostpartal women and the data for postpartal women were collected for two different studies with no attempt to match subjects on sociodemographic variables. Specimens were analyzed on different days, and different databases were used to store data. These methodologic differences preclude statistical comparisons between the two groups. However, data from healthy, nonpostpartal women are included as a reference cohort.

All women in the postpartal group had vaginal deliveries and were excluded from the study if they had chronic medical and psychiatric problems, any known infection, or hemoglobin levels of less than 8 mg/dl after delivery, admitted to being drug or alcohol abusers, or had clinical evidence of drug and/or alcohol abuse or infants who demonstrated clinical signs of maternal drug abuse or human immunodeficiency virus. Any of these conditions could have independently influenced immune status. Mothers who met study criteria were asked if they wished to participate, and informed consent was obtained in accordance with the guidelines of the University of Pennsylvania Institutional Review Board.

Methods. Data were collected at the following four time points: within 24 h of delivery and at 1, 2, and 4 months postpartum. These periods were chosen as being the most likely, based on the existing literature, to provide information about when the return to prepregnancy immune function occurs (1, 5, 15, 22). Peripheral blood samples were obtained from mothers, and complete blood counts and differentials were performed. Additionally, cell phenotypes, including CD3, CD4, CD8, $CD3^+/CD57^+$, CD11b, $CD4^+/CD29^+$, $CD4^+/CD45RA^+$, and HLA class II Ia, were determined on lymphocytes; the B-cell marker (CD20), monocyte markers (including CD11b, CD14, and Ia), and NK cell markers ($CD3^-/CD16^+$, $CD3^-/CD56^+$, and $CD3^-/CD57^+$) were also determined (Table 1).

Complete blood counts and differentials and cell phenotyping were also performed for nonpostpartal, healthy women (17). The cell phenotypes that were determined with this group included CD3, CD4, CD8, CD11b, $CD4^+/CD29^+$, CD20, CD14, and Ia on lymphocytes and monocytes.

Cell phenotypes in circulating peripheral blood samples were measured by fluorescence-activated flow cytometry. Briefly, 0.02 ml of 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 per liter, 1.00 g of potassium bicarbonate per liter, and 0.037 g of EDTA per liter, maintained at a pH of 7.3) was added to each sample and incubated for 1.5 min to lyse erythrocytes. This was followed by a final wash with phosphate-buffered saline. The samples were then fixed by the addition of 0.5 ml of 1% paraformaldehyde prepared in phosphate-buffered saline (pH 7.3). Samples were stored at 4°C in the dark until 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 output was used for all immunophenotypic studies. The instrument was calibrated for forward and side scatter detection, as well as for fluorescence detection employing standard DNA check microspheres (Coulter Immunology, Hialeah, Fla.) prior to all sample evaluations. 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

TABLE 2. Leukocyte and absolute cell counts of samples from postpartal women

Cell type	Mean count ± SE ($10^3/mm^3$) at[a]:				P^b
	Delivery	1 mo postpartum	2 mo postpartum	4 mo postpartum	
Leukocyte	11.24 ± 0.42 (10.4, 12.1)	6.39 ± 0.24 (5.9, 6.9)	6.11 ± 0.20 (5.7, 6.5)	6.21 ± 0.25 (5.7, 6.7)	<0.001
Lymphocyte	1.97 ± 0.09 (1.8, 2.2)	2.35 ± 0.11 (2.1, 2.6)	2.22 ± 0.08 (2.1, 2.4)	2.35 ± 0.10 (5.7, 6.7)	0.003
Eosinophil	0.15 ± 0.01 (0.12, 0.18)	0.21 ± 0.03 (0.15, 0.26)	0.18 ± 0.02 (0.14, 0.22)	0.41 ± 0.03 (0.13, 0.21)	0.28
Neutrophil	8.35 ± 0.42 (7.5, 9.2)	3.33 ± 0.21 (2.9, 3.8)	3.23 ± 0.17 (2.9, 3.6)	3.22 ± 0.18 (2.8, 3.6)	<0.001
Monocyte	0.68 ± 0.05 (0.58, 0.79)	0.44 ± 0.02 (0.39, 0.48)	0.43 ± 0.02) 0.39, 0.48)	0.41 ± 0.03 (0.36, 0.47)	<0.001
Basophil	0.07 ± 0.01 (0.05, 0.09)	0.05 ± 0.01 (0.04, 0.06)	0.04 ± 0.00 (0.03, 0.05)	0.04 ± 0.01 (0.02, 0.06)	0.01

[a] Data in parentheses are confidence limits.
[b] Repeated-measures ANOVA was performed by BMDP software.

TABLE 3. Percentages of cell types in samples from postpartal women

Cell type	Mean ± SE (%) at[a]:				P^b
	Delivery	1 mo postpartum	2 mo postpartum	4 mo. postpartum	
Lymphocyte	19.00 ± 1.22 (16.5, 21.5)	37.95 ± 1.59 (34.7, 41.1)	37.23 ± 1.37 (34.4, 39.9)	38.93 ± 1.60 (35.7, 42.1)	<0.001
Eosinophil	1.48 ± 0.15 (1.1, 1.8)	3.31 ± 0.40 (2.5, 4.1)	3.05 ± 0.35 (2.3, 3.7)	2.85 ± 0.36 (2.1, 3.6)	<0.001
Neutrophil	72.58 ± 1.50 (69.6, 75.6)	50.77 ± 1.78 (47.2, 54.3)	51.69 ± 1.47 (48.7, 54.6)	50.74 ± 1.59 (47.5, 53.9)	<0.001
Monocyte	6.16 ± 0.42 (5.3, 6.9)	6.98 ± 0.32 (6.3, 7.6)	7.21 ± 0.37 (6.4, 7.9)	6.67 ± 0.40 (5.8, 7.5)	<0.07
Basophil	0.65 ± 0.10 (0.46, 0.85)	0.75 ± 0.09 (0.56, 0.92)	0.72 ± 0.08 (0.55, 0.87)	0.63 ± 0.08 (0.47, 0.78)	0.60

[a] Data in parentheses are confidence limits.
[b] Repeated-measures ANOVA was performed by BMDP software.

than 2% positive for CD14, while the monocyte cluster must be greater than 80% positive for CD14. A higher-level gating criterion for monocytes (e.g., 90% positive for CD14) was originally used. However, for approximately one-third of women, monocyte data would not have been interpretable given this criterion. The 80% cutoff was 1 standard deviation below the mean obtained for postpartum women and allowed for relative assurance that all monocytes were counted. In other studies, monocyte clusters with lower levels of CD14 expression have also been demonstrated (11).

Fluorescence data for specific markers were obtained in two-color format, with quadrants being established where the isotype control samples yielded greater than 95% fluorescence signals within the red negative-green negative quadrant (quadrant 3). Both the percentages of positive cells and mean channel fluorescence values were recorded from appropriate quadrants for lymphocytes and monocytes with absolute values obtained from complete blood count and differential data obtained with each sample with an STKS (Coulter Electronics). Although mean channel fluorescence values were obtained, recorded, and analyzed for a subset of both lymphocyte and monocyte markers, no significant differences were found over time in mean channel fluorescence values; therefore, mean channel fluorescence values are excluded from any further discussion here.

RESULTS

Repeated-measured analysis-of-variance (ANOVA) models were fit to all parameters by using the BMDP program (see Tables 2 through 5). The models used the actual time since delivery in the fit, and models were evaluated for both quadratic and linear fits. Based on the significance of the quadratic coefficient and the size of the residuals, the results of the linear models are presented.

Leukocyte counts. The total leukocyte count in postpartal women was elevated after delivery but dropped significantly by 1 month ($P < 0.0001$). There was no significant difference in the absolute eosinophil count over the first 4 postpartal months ($P = 0.28$), but the percentage of eosinophils among total leukocytes increased significantly ($P < 0.001$), with the percentage of eosinophils among total leukocytes increased significantly ($P < .001$), with the percentage of eosinophils increasing from delivery to 1 month, remaining stable until 2 months, and then dropping slightly by 4 months (Tables 2 and 3). Both the absolute lymphocyte count ($P = 0.003$) and percentage of lymphocytes ($P < 0.001$) increased significantly from delivery to 1 month postpartum and remained stable thereafter. The absolute monocyte count ($P < 0.001$) dropped slightly from delivery until 1 month postpartum and then remained stable, although the change in the percentage of monocytes was not statistically significant ($P = 0.07$). The absolute number of basophils dropped slightly from delivery until 1 month postpartum and then remained stable ($P = 0.01$), but the percentage of basophils did not differ over time ($P = 0.60$).

Lymphocyte cell subsets. The levels of some lymphocyte cell subsets remained stable over the first 4 postpartal months; these included $CD4^+/CD45RA^+$ (an inducer-suppressor cell) and Ia on lymphocytes (Tables 4 and 5), both of which showed no significant change either in cell numbers or in the percentage of cells in peripheral blood. Other lymphocyte subsets, including CD3 (pan-T cell) and CD4 (helper T cell), had significant increases in the absolute numbers of cells but not in the percentages of these cells.

The levels of other lymphocyte cell subsets increased over time. For example, the levels of $CD4^+/CD29^+$ (a memory cell population that provides help to B cells for immunoglobulin synthesis and to T cells for cell-mediated immunity) rose sig-

TABLE 4. Percentages of lymphocyte, monocyte, and NK cell subsets in samples from postpartal women

Cell subset	Mean ± SE (%) at[a]:				P^b
	Delivery	1 mo postpartum	2 mo postpartum	4 mo postpartum	
CD3	83.11 ± 0.83 (81.4, 84.7)	83.46 ± 0.74 (81.9, 84.9)	83.05 ± 0.91 (81.2, 84.8)	82.97 ± 0.83 (81.3, 84.6)	0.91
CD4	48.19 ± 1.60 (44.9, 51.4)	48.94 ± 1.16 (46.6, 51.3)	49.16 ± 1.10 (46.9, 51.4)	50.20 ± 1.12 (47.8, 53.5)	0.68
CD8	20.95 ± 1.03 (18.8, 23.1)	22.67 ± 1.26 (20.1, 25.1)	23.31 ± 0.99 (21.3, 25.3)	20.42 ± 1.06 18.3, 22.5)	0.01
$CD3^+/CD57^+$	9.84 ± 0.97 (7.8, 11.8)	11.67 ± 1.00 (9.6, 13.6)	11.56 ± 0.88 (9.7, 13.3)	10.31 ± 0.81 (8.7, 11.9)	0.009
$CD4^+/CD29^+$[c]	42.34 ± 1.83 (38.7, 46.0)	49.23 ± 1.63 (45.9, 52.5)	51.45 ± 1.41 (48.6, 54.3)	50.65 ± 1.41 (47.8, 53.5)	<0.0001
$CD4^+/CD45RA^+$[c]	45.87 ± 2.19 (41.5, 50.2)	44.11 ± 1.89 (40.3, 47.9)	44.7 ± 1.72 (40.7, 47.6)	41.91 ± 1.82 (38.2, 45.6)	0.34
CD11b lymphocyte	8.64 ± 0.84 (5.9, 10.3)	10.49 ± 0.98 (8.5, 12.4)	10.06 ± 0.85 (8.3, 11.7)	8.29 ± 0.85 (6.5, 9.9)	0.02
Ia lymphocyte	14.22 ± 0.92 (12.4, 16.1)	12.49 ± 0.71 (11.1, 13.9)	13.46 ± 0.72 (12.0, 14.9)	13.06 ± 0.93 (13.8, 18.8)	0.56
CD20	9.22 ± 0.61 (7.9, 20.5)	8.69 ± 0.70 (7.3, 10.1)	9.12 ± 0.60 (7.9, 10.3)	9.53 ± 0.75 (8.0, 11.0)	0.53
$CD3^-/CD16^+$	4.81 ± 0.35 (4.1, 5.5)	5.92 ± 0.42 (5.1, 6.7)	5.63 ± 0.45 (4.7, 6.5)	5.61 ± 0.52 (4.6, 6.7)	0.009
$CD3^-/CD56^+$	7.33 ± 0.76 (5.8, 8.9)	6.70 ± 0.72 (5.2, 8.1)	6.42 ± 0.78 (4.8, 7.9)	6.35 ± 0.72 (4.9, 7.8)	0.55
$CD3^-/CD57^+$	2.43 ± 0.29 (1.9, 3.0)	3.26 ± 0.37 (2.5, 4.0)	3.19 ± 0.37 (2.5, 3.9)	3.05 ± 0.40 (2.3, 3.9)	<0.0001
CD14	92.29 ± 1.06 (90.14, 94.4)	89.89 ± 1.30 (87.27, 92.50)	90.73 ± 1.41 (87.90, 93.56)	88.52 ± 1.53 (85.44, 91.58)	0.42
CD11b monocyte	95.02 ± 1.66 (84.11, 90.72)	90.60 ± 2.29 (76.89, 86.87)	96.04 ± 3.14 (81.79, 89.39)	95.02 ± 2.93 (78.79, 87.2)	0.13
Ia monocyte	91.66 ± 1.72 (80.74, 88.84)	95.21 ± 2.08 (81.53, 91.45)	97.17 ± 2.05 (83.91, 93.28)	96.41 ± 1.54 (81.54, 89.64)	0.07

[a] Data in parentheses are confidence limits.
[b] Repeated-measures ANOVA was performed by BMDP software.
[c] Percentage of total $CD4^+$ cells which coexpress CD29 or CD45RA.

TABLE 5. Absolute numbers of lymphocyte, monocyte, and NK cell subset in samples from postpartal women

Cell subset	Mean no. ± SE (10^3/mm^3) at[a]:				P^b
	Delivery	1 mo postpartum	2 mo postpartum	4 mo postpartum	
CD3	1.63 ± 0.08 (1.5, 1.8)	1.95 ± 0.09 ± (1.8, 2.1)	1.83 ± 0.06 (1.7, 1.9)	1.94 ± 0.08 (1.8, 2.1)	0.01
CD4	0.95 ± 0.05 (0.8, 1.0)	1.14 ± 0.06 (1.0, 1.3)	1.08 ± 0.04 (1.0, 1.2)	1.18 ± 0.06 (1.1, 1.3)	0.009
CD8	0.42 ± 0.03 (0.4, 0.5)	0.54 ± 0.04 (0.5, 0.6)	0.53 ± 0.03 (0.5, 0.6)	0.49 ± 0.04 (0.4, 0.6)	0.0001
$CD3^+/CD57^+$	0.05 ± 0.01 (0.03, 0.06)	0.08 ± 0.01 (0.06, 0.10)	0.08 ± 0.01 (0.06, 0.10)	0.08 ± 0.02 (0.05, 0.12)	0.006
$CD4^+/CD29^{+c}$	0.83 ± 0.05 (0.7, 0.9)	1.16 ± 0.07 (1.0, 1.3)	1.15 ± 0.06 (1.0, 1.3)	1.20 ± 0.07 (1.1, 1.3)	<0.001
$CD4^+/CD45RA^{+c}$	0.92 ± 0.06 (0.8, 1.0)	1.05 ± 0.08 (0.9, 1.2)	0.53 ± 0.03 (0.9, 1.1)	1.00 ± 0.07 (0.9, 1.1)	0.07
CD11b lymphocyte	0.17 ± 0.02 (0.1, 0.2)	0.25 ± 0.03 (0.2, 0.3)	0.23 ± 0.02 (0.2, 0.3)	0.20 ± 0.03 (0.1, 0.3)	0.001
Ia lymphocyte	0.29 ± 0.02 (0.2, 0.3)	0.30 ± 0.02 (0.2, 0.3)	0.31 ± 0.03 (0.3, 0.4)	0.32 ± 0.03 (0.3, 0.4)	0.21
CD20	0.19 ± 0.02 (0.1, 0.2)	0.21 ± 0.02 (0.2, 0.3)	0.21 ± 0.02 (0.2, 0.25)	0.23 ± 0.02 (0.20, 0.25)	0.32
$CD3^-/CD16^+$	0.09 ± 0.01 (0.01, 0.1)	0.14 ± 0.01 (0.1, 0.2)	0.13 ± 0.01 (0.1, 0.2)	0.14 ± 0.02 (0.09, 0.2)	<0.0001
$CD3^-/CD56^+$	0.14 ± 0.02 (0.1, 0.2)	0.16 ± 0.02 (0.1, 0.2)	0.15 ± 0.02 (0.1, 0.2)	0.16 ± 0.03 (0.1, 0.2)	0.33
$CD3^-/CD57^+$	0.05 ± 0.01 (0.04, 0.06)	0.08 ± 0.01 (0.06, 0.1)	0.08 ± 0.01 (0.06, 0.10)	0.08 ± 0.02 (0.04, 0.1)	<0.001
CD14	0.64 ± 0.05 (0.5, 0.7)	0.39 ± 0.02 (0.3, 0.4)	0.40 ± 0.02 (0.35, 0.4)	0.37 ± 0.03 (0.3, 0.4)	0.003
Cd11b monocyte	0.61 ± 0.05 (0.5, 0.7)	0.36 ± 0.02 (0.3, 0.4)	0.38 ± 0.02 (0.3, 0.4)	0.35 ± 0.03 (0.3, 0.4)	0.0001
Ia monocyte	0.59 ± 0.05 (0.5, 0.7)	0.38 ± 0.02 (0.3, 0.4)	0.38 ± 0.02 (0.3, 0.4)	0.36 ± 0.03 (0.3, 0.4)	0.0001

[a] Data in parentheses are confidence limits.
[b] Repeated-measures ANOVA was performed by BMDP software.
[c] Of total $CD4^+$ cells which coexpress CD29 or CD45RA.

nificantly both in cell numbers ($P < 0.001$) and cell percentages ($P < 0.001$) from delivery until 2 months postpartum and then plateaued (Fig. 1).

The levels of other cell subsets increased in the early postpartum and then decreased. For example, the levels of CD8 (a cytotoxic T cell) increased slightly from delivery until 2 months postpartum and then dropped again by 4 months postpartum (Fig. 2) so that there was a significant change in the CD8 percentage ($P = 0.0001$) and in the absolute cell count ($P = 0.01$). Similarly, the level of CD11b expressed on lymphocytes (C3bi receptor) rose from delivery until 1 month postpartum, where it remained stable until 4 months, when it again decreased in the percentage of cells ($P < 0.001$) and in absolute cell count ($P = 0.02$). $CD3^+/CD57^+$ levels (a measure of total activated T cells) changed significantly both in cell number ($P = 0.006$) and in cell percentage ($P < 0.0009$), with a rise in both absolute numbers and percentages from delivery until 1 month postpartum. The levels of CD20, the only B-lymphocyte cell subset that was examined, did not change over the first 4 postpartal months in either the absolute number of cells ($P = 0.32$) or the percentage of cells ($P = 0.53$).

NK cells. NK cell ($CD3^-/CD16^+$, $CD3^-/CD56^+$, and $CD3^-/CD57^+$) levels were examined. $CD3^-/CD16^+$ levels rose from delivery until 1 month postpartum and then plateaued in both cell numbers ($P = <0.0001$) and percentage of cells ($P = 0.009$) (Fig. 3); $CD3^-/CD57^+$ levels also rose from delivery until 1 month postpartum, and then both the number of cells ($P < 0.0001$) and the percentage of cells ($P < 0.0001$) remained stable (Fig. 4). $CD3^-/CD56^+$ levels were stable over the first 4 postpartal months (Tables 4 and 5).

Monocyte subsets. CD14 (a monocyte lipopolysaccharide receptor) levels decreased slightly from delivery until 1 month postpartum and then remained stable so that there was a significant difference in cell number ($P = 0.003$) but not in percentage of cells ($P = 0.42$) over the first 4 postpartal months. The levels of CD11b expressed on monocytes dropped from delivery until 1 month postpartum, and there was a significant difference in cell number ($P = 0.0001$) but not in percentage of cells ($P = 0.13$) over the first 4 postpartal months. The levels of Ia on monocytes (a polymorphic class II antigen) did not significantly differ in cell percentage ($P = 0.07$), but cell numbers increased slightly from delivery until 1 month postpartum and then plateaued ($P = 0.0001$) (Tables 4 and 5).

Postpartal values at 4 months compared to other reference ranges. The results obtained for each cell subset at 4 months postpartum were compared with reported norms in the literature and with data from healthy nonpostpartal women from whom lymphocyte, monocyte, and NK cell subsets were also obtained and analyzed in the same laboratory (17). For these comparisons, data for 64 mothers who delivered preterm VLBW infants in our larger study are presented along with the data for the 51 mothers of healthy term infants from this study. Seven studies that included reference ranges of peripheral blood subsets, determined by flow cytometry, from healthy individuals are also reported (4, 6, 9, 12, 13, 22, 24). Addition-

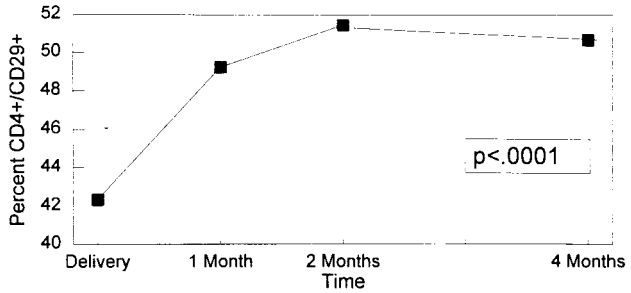

FIG. 1. Changes in $CD4^+/CD29^+$ percentage over time.

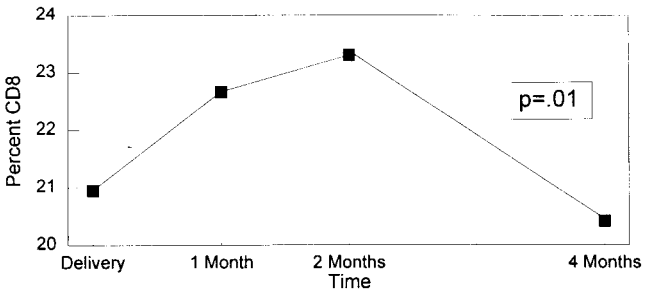

FIG. 2. Changes in CD8 percentage over time.

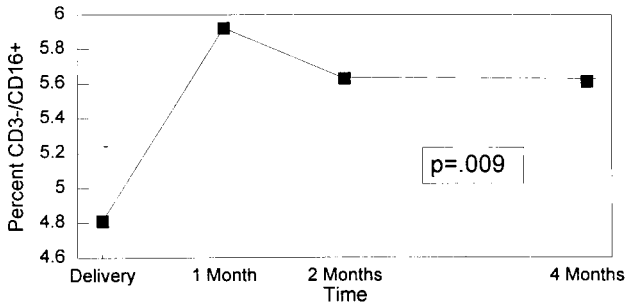

FIG. 3. Changes in $CD3^-/CD16^+$ percentage over time.

ally, peripheral blood lymphocyte subsets of healthy women are being analyzed in the same immunology laboratory in which blood samples from our ongoing study are being analyzed, which provides an excellent measure of quality control and an eighth study for comparison (17).

In Tables 6 through 8, sample sizes, genders of donors, and ages are included for direct comparisons. All of the studies had groups that included African-Americans and Caucasians, except for one group that was solely comprised of Caucasians (4) (Table 6).

At 4 months postpartum, both the mean and median ranges for the CD3 percentage and absolute cell count in peripheral blood are somewhat higher in postpartal women than in reported normal samples, although they are within the normal reference range (61 to 85%) cited by Reichert and colleagues (20). The mean and median percentages of CD4, a helper T-cell subset, are similar to reported ranges for both men and women (9, 13, 17, 22, 24). However, both mean and median percentages of CD8, a suppressor T-cell subset, are lower in the study sample than in any of the other studies (4, 12, 13, 22). However, the CD8 levels of nonpostpartal women which were also measured in our laboratory (17) were comparable to the values obtained from postpartal women. The postpartal women at 4 months had a mean of 20.0%. Mothers of VLBW infants had a slightly higher value (23.2%), and the healthy nonpostpartal women who were also tested in our laboratory had a mean of 22.7% (17) (Tables 6 and 7).

There is a wide variability in the range of $CD3^-/CD16^+$ levels reported in the literature, but the levels of this NK cell subset in postpartal women were lower than those reported for healthy subjects (9), healthy controls from our own laboratory (17), depressed patients (6), and Caucasian men (4). There were also lower $CD4^+/CD45RA^+$ levels, a suppressor-inducer T-cell subset, in postpartal women than in comparison groups (4). At 4 months postpartum, monocyte rates approximated or were slightly higher than those values obtained from healthy,

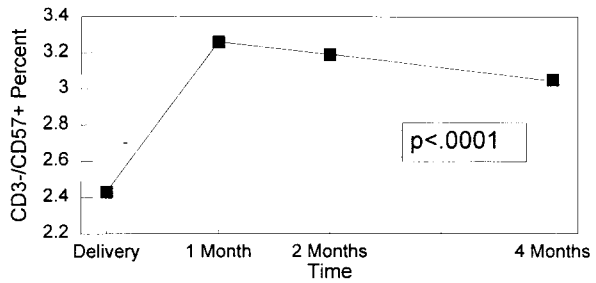

FIG. 4. Changes in $CD3^-/CD57^+$ percentage over time.

TABLE 6. Median percentages of lymphocyte populations in peripheral blood samples of adults

Cell subset	Median (%)[a] in samples from:				
	Postpartal mothers of term infants[b] (51)	Postpartal mothers of VLBW infants (64)	Healthy nonpostpartal women (112)	Men and women[c]	Caucasian men (15)
CD3	82.5	83.1		75	81
CD4	48.3	48.6	47	48	49
CD8	20.4	20.5	22.1	32	32
CD20	8.6	8.9	10.6		12
$CD4^+$ / $CD45RA^+$	39.2	34.4			65
$CD3^-/CD56^+$	5.2	5.1			18

[a] Data in the first two columns are from this study. Data in the other columns are from references 17, 12, and 4, respectively. Numbers in parentheses indicate the numbers of individuals.
[b] At 4 months postpartum.
[c] Ages, 18 to 70 years old.

nonpostpartal women studied in the same laboratory as the study subjects (17). CD20 (a B-cell subset) rates in postpartal women were lower than those reported for other groups (4, 13, 17), and the levels of CD11b on lymphocytes in postpartal women were lower than those in the healthy, nonpostpartal women studied in the same laboratory (17).

DISCUSSION

Leukocytosis seen after delivery is attributable to the physiologic stress of labor, which increases the already high leukocyte count of the pregnant woman. Leukocytosis is primarily attributable to neutrophilia (5) and generally resolves within the first postpartal week (1). Eosinophil levels are known to decrease late in pregnancy (5), so the slight increase from delivery to 1 month postpartum probably indicates a return to prepregnancy status. Lymphocytopenia, which the women had at delivery but which was resolved by 1 month, has also been reported before and has been attributed to the lympholytic effect of elevated cortisol levels at delivery (1).

The levels of some NK cell and lymphocyte subsets, such as $CD3^-/CD56^+$ and $CD4^+/CD45RA^+$, remained stable over the first 4 postpartal months. However, not all researchers have demonstrated the same postpartal stability for these subsets. Stagnaro-Green and colleagues (22) found increased $CD4^+/CD45RA^+$ levels in the postpartum period, although these levels remained steady in the postpartal women we studied. $CD4^+/CD45RA^+$ is only beginning to be examined during pregnancy and the postpartum, but its levels are diminished in individuals with established autoimmune disease, such as multiple sclerosis and systemic lupus erythematosus. Further work is needed to determine if changes in this subset can provide important clinical information about postpartal exacerbations to autoimmune disease. Likewise, Stagnaro-Green and colleagues (22) reported a postpartal decline in CD4 levels (from 48% at 3 months to 46% at 6 months, as compared to our 50.2% at 4 months); in our subjects, although there was actually a significant increase in the absolute number of CD4, the percentage of these cell subsets in peripheral blood remained stable over the first 4 postpartal months.

CD8 percentages and numbers rose transiently and then decreased by 4 months. Additionally, CD8 levels in both postpartal subjects and nonpostpartal women from our laboratory were considerably lower than the percentages of CD8 reported in a number of other studies (4, 12, 22). However, in another

TABLE 7. Mean lymphocyte percentages in peripheral blood samples of adult women

Cell subset	Mean ± SEM (%)[a] in samples from:							
	Mothers of VLBW infants[b] (64)	Postpartal women[b] (51)	Women[c] (12)	Postpartal women[d] (28)	Women (32)	Postpartal women[b] (22)	Women[e] (60)	Women[f] (32)
CD3	82.1 ± 1.2	82.9 ± 0.8	78.82				70.5	76.3 ± 2.1
CD4	47.7 ± 1.2	50.2 ± 1.1	46.1 ± 0.8	48.0 ± 1.4	53.2 ± 1.3	38.7 ± 3.4	45.9	45.9 ± 1.2
CD8	23.2 ± 1.2	20.4 ± 1.1	22.7 ± 0.8	30.5 ± 1.2	26.9 ± 0.9	24.2 ± 5.9		
CD14	93.2 ± 0.6	88.5 ± 1.5	91.4 ± 2.5					
CD11b lymphocytes	8.7 ± 1.9	8.3 ± 0.9	16.3 ± 2.8					
CD11b monocytes	86.3 ± 1.8	95.0 ± 1.5	87.8 ± 2.8					
$CD3S^-/CD16^+$	6.2 ± 0.4	5.6 ± 0.5	10.3 ± 1.3			11.6 ± 2.8	16.1	
$CD3^-/CD57^+$	2.7 ± 0.3	3.1 ± 0.4	5.4 ± 0.9			16.4 ± 5.0		
CD20	9.4 ± 0.5	9.5 ± 0.8	11.6 ± 0.5			16.2 ± 2.0		
Ia lymphocytes	13.7 ± 1.4	13.1 ± 0.9	14.2 ± 1.2					
Ia monocytes	91.3 ± 1.4	96.4 ± 1.5	88.3 ± 4.3					

[a] Data in the first two columns are from this study. Data in the other columns are from references 17, 22, 22, 13, 9, and 24, respectively. Numbers in parentheses indicate the numbers of individuals.
[b] At 4 months postpartum.
[c] Age, 27 years old.
[d] At 3 months postpartum.
[e] Ages, 20 to 99 years old.
[f] Ages, 17 to 19 years old.

study that also measured the levels in postpartal women at 4 months, comparable values of CD8 were reported (13). These differences in CD8 levels between studies may be due to the time during the postpartal period that CD8 was measured. In both this study and the work of Stagnaro-Green and associates (22), CD8 levels have been demonstrated to increase over time in postpartal women. Perhaps there is an increase in cytotoxic activity in the early postpartal period.

A comparative study of CD8 values in pregnant women (22.8% ± 5.6%) versus nonpregnant women (28.0% ± 8.9%) indicates that the lower postpartum CD8 values reported here, which are similar to the CD8 values reported for pregnant women elsewhere (18), are consistent with other observations of alterations in immune function during pregnancy which extend into the postpartum period.

The increases in the percentages and numbers from delivery until 1 month postpartum for some of the other lymphocyte cell subsets (e.g., $CD3^+/CD57^+$ and $CD4^+/CD29^+$) as well as similar increases in NK cell subsets ($CD3^-/CD16^+$ and $CD3^-/CD57^+$) from delivery until 1 month postpartum may also indicate a natural mechanism of increased immune response in the early postpartal period in healthy women. However, because the $CD3^-/CD16^+$ and $CD3^-/CD57^+$ levels in the postpartal women in this study were so much lower than the percentages of NK cells found in nonpostpartal groups, it appears more likely that even if the numbers of immune cell subsets increase dramatically in the first postpartal month to augment the postpartal woman's ability to mount a defense against pathogens, women are still recovering from the relative immunosuppression of pregnancy at least during the first 4 postpartal months.

The reference ranges provided for postpartal women in this study demonstrate that there are rapid changes in the early postpartal period in the percentages of some immune cell subsets present in peripheral blood. However, the lower levels of many cell subsets at 4 months in postpartal women compared to those in nonpostpartal women appear to indicate that recovery from the immunosuppression of pregnancy occurs slowly over the first few postpartal months. Postpartal women do appear to have values for many cell subsets that differ from those of other populations; therefore, the reference ranges in Tables 2 through 5 may be helpful in clinical decision making. Additionally, this study provides important new information about the expected levels of monocytes in postpartal women. Because the criteria for gating monocytes are being established, the criteria and values provided here may be particularly helpful in determining whether a particular postpartal women has normal percentages or cell counts of monocyte subsets.

The findings of this study indicate that the immune status of postpartal women slowly returns to prepregnancy levels over the first 4 postpartal months and that there are normal differences over that period between the immune status of postpartal women and that of other healthy women. Because the postpartal period is a time when changes are occurring in immune status, in order to be able to determine if postpartal

TABLE 8. Absolute lymphocyte counts in peripheral blood samples of adults

Cell subset	Mean absolute count ± SEM ($10^3/mm^3$)[a] in samples from:			
	Postpartal mothers of VLBW infants (64)	Postpartal mothers at 4 months (51)	Depressed patients (14)	Healthy controls (29)
CD3	1.86 ± 0.07	1.9 ± 0.08	1.52 ± 0.12	1.57 ± 0.20
CD4	1.09 ± 0.05	1.2 ± 0.06	0.92 ± 0.06	0.93 ± 0.16
CD8	0.53 ± 0.03	0.5 ± 0.04	0.44 ± 0.05	0.48 ± 0.06
$CD3^-/CD16^+$	0.13 ± 0.01	0.14 ± 0.02	0.18 ± 0.02	0.26 ± 0.07
$CD3^+/CD57^+$	0.06 ± 0.01	0.08 ± 0.02	0.14 ± 0.02	0.31 ± 0.08

[a] Data in the first two columns are from this study. Data in the other two columns are from reference 6. Numbers in parentheses indicate the numbers of individuals.

women who are experiencing health problems have normal immune values, it is clearly necessary to compare individual values with other reference values obtained from postpartum women.

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