

Postpartum maternal blood helper T (CD3⁺CD4⁺) and cytotoxic T (CD3⁺CD8⁺) cells: correlations with iron status, parity, supplement use, and lactation status¹⁻³

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ABSTRACT Iron deficiency reduces T cell counts; however, iron sufficiency is difficult to maintain during pregnancy and to reestablish in the early postpartum period. This cross-sectional study examined relations among postpartum maternal iron status, parity, lactation, supplement use, and maternal blood T cell populations. Sixty lactating and 41 nonlactating postpartum (NLPP) women at 1–2 wk and 1, 2, 4, or 8 mo postpartum and 13 nulliparous women were studied. Among multiparous women, multiple linear regression showed that relative percentages and absolute numbers of CD3⁺CD8⁺ cells were correlated positively with maternal serum transferrin saturation. In a separate multiple linear regression model, multiparous NLPP women who did not use multivitamin and mineral supplements had lower CD3⁺CD4⁺ cell percentages in the first month postpartum than did nulliparous control women. Lactating women who used supplements, however, had reduced CD3⁺CD4⁺ percentages 4–8 mo postpartum compared with control women. CD3⁺CD4⁺ percentages did not differ among control women, NLPP women who used supplements, or lactating women who did not use them. These results suggest that nutritional factors such as maternal iron status and use of dietary supplements play a role in a mother's postpartum immune status. *Am J Clin Nutr* 1998;67:897–904.

KEY WORDS Lactation, postpartum period, humans, immunology, iron, transferrin saturation, T cell, multivitamin and mineral supplements, iron deficiency, CD3⁺CD4⁺ cells, CD3⁺CD8⁺ cells, iron status

INTRODUCTION

Pregnant women require more iron for a healthy pregnancy than is provided by the average woman's iron reserves plus the average amount of iron consumed in the US diet. This fact led the Food and Nutrition Board to recommend that women take a daily 15-mg Fe supplement through the second and third trimesters of pregnancy (1). In clinical practice, supplemental iron often is provided in prenatal formulations containing other vitamins and minerals.

After delivery, the time required for iron stores to become replete is rarely addressed. In the opinion of one author, "several months or even years are required [to accumulate enough iron for another pregnancy] with our present kind of diet" (2). The average nonpregnant, nonlactating, healthy woman is estimated to

have 300 mg of stored iron reserves (3). The net deficit between absorbed and required iron through pregnancy is estimated to be 440 mg (2). Although lactating women do not have menstrual blood iron losses postpartum (as a result of lactational amenorrhea), they require 1.1 mg Fe/d to compensate for basal (0.9 mg/d) and milk (0.2 mg/d) iron losses (4, 5). Given the estimated average iron density of the US diet, lactating women consume ≈15 mg Fe/d with an intake of 9204 kJ/d, or 2200 kcal/d (6). If women in the early postpartum period absorb twice the 1.5–2.2 mg Fe/d of the typical nonpregnant, nonlactating woman (1), ≈3.7 mg Fe can be absorbed daily from this diet. Under these conditions, a lactating US woman with average iron stores at the onset of pregnancy (and not using iron supplements) requires ≈2 mo to recover from a predicted iron deficiency state and another 4 mo for iron stores to return to prepregnancy replete amounts (stored – deficit)/(daily absorption – requirements) = (300 mg – 440 mg)/(3.7 mg/d – 1.1 mg/d) = 54 d. Under the same conditions, a lactating woman with low iron stores before pregnancy could require 5–6 mo to recover from iron deficiency and an additional 4 mo or more for iron stores to become replete.

Iron-deficient adults have significantly reduced peripheral blood helper T (CD4⁺) cell counts (≈60% of those of control subjects) that rise to normal values after iron repletion (7). In vitro evidence indicates that cytotoxic T (CD8⁺) cells and a subset of CD4⁺ cells are the lymphocytes most sensitive to limited iron availability (8). Given the putative periods of iron depletion and repletion around the time of delivery, responsive changes would be expected in maternal T cell numbers, as seen in previous studies of iron-deficient children and animal models of iron deficiency (9). This study evaluated how maternal iron status, lactation, and supplement use are correlated with relative fre-

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² Supported by National Institutes of Health grant RO1 HD21049-06 and a National Science Foundation fellowship.

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Received September 3, 1997.

Accepted for publication October 31, 1997.

quencies (percentages) and absolute counts of maternal peripheral blood T cells (CD3⁺), cytotoxic T cells (CD3⁺CD8⁺), and helper T cells (CD3⁺CD4⁺) in the first 8 mo postpartum.

SUBJECTS AND METHODS

Subjects

Sixty-three lactating, 19 nonlactating postpartum (NLPP), and 14 nulliparous control women were recruited through the prenatal care clinic at Bassett Healthcare Center Hospital in Cooperstown, NY, from May through August 1993. Because 71% of women delivering their infants at Bassett Hospital start breast-feeding and most of the NLPP women there use hormonal birth control (an exclusion criterion), 25 additional NLPP women were recruited from prenatal classes in the Houston area through the US Department of Agriculture Children's Nutrition Research Center from May through August 1994. Seven subjects were later excluded from the data analysis (*see* Results), giving a final sample size of 60 lactating, 41 NLPP, and 13 nulliparous subjects.

Age, education, body mass index (BMI, in kg/m²), and parity data are presented in **Table 1**. All participants were white, to remain consistent with the 99% white demographics of the Cooperstown area. The study protocol was approved by the human subject use institutional review boards of Cornell University, Bassett Hospital, Baylor College of Medicine, St Luke's Episcopal Hospital, and the University of Texas Medical Branch.

The study had a cross-sectional design, with each woman participating only once between 1 and 2 wk or ≈ 1 (± 3 d), 2 (± 6 d), 4 (± 12 d), or 8 (± 18 d) mo postpartum. Lactating women at ≤ 4

mo postpartum were fully breast-feeding (infants received less than one non-breast-milk feeding per week). Eight months postpartum, lactating women were breast-feeding four or more times per day. Only one mother was fully breast-feeding at 8 mo. Potential subjects were excluded for self-reported hormonal birth control use within the past month, illness in the past 2 wk, or a history of chronic endocrine or immune system disorders.

Sampling

Fasting blood samples were drawn between 0900 and 1100. An Na-EDTA-preserved blood sample was divided for flow cytometric staining (described below) and hematologic analyses in the hospital's clinical laboratory. Hematologic analyses included a complete blood count, differential white cell count, and measurement of the erythrocyte sedimentation rate (ESR). A sample without preservative was allowed to clot for 1–3 h at room temperature and centrifuged at $400 \times g$ for 10 min at room temperature to obtain serum. Spot urine samples were screened with a urine pregnancy test to exclude pregnant subjects. During her visit, each subject completed a questionnaire that included items on maternal parity and current use of multivitamin and mineral supplements (including prenatal or over-the-counter formulations).

Monoclonal antibodies

Monoclonal antibodies for flow cytometric analysis were purchased from the manufacturer conjugated to peridin-chlorophyll (PerCP), fluorescein isothiocyanate (FITC), or phycoerythrin (PE). The antibody specificities used were as follows: CD45-FITC/CD14-PE, for gating of lymphocytes compared with monocytes; CD3-PerCP, a T cell marker (Becton Dickinson

TABLE 1
Subject characteristics¹

Group	Age y	BMI kg/m ²	Parity	Education y	Supplement use ²
Control (n = 13)	31.0 \pm 6.5	26.2 \pm 7.7	—	15.8 \pm 2.5	7
Lactating					
1–2 wk (n = 8)	30.5 \pm 2.9	27.1 \pm 3.2	2.3 \pm 1.2 [2] ³	14.8 \pm 2.5	7
1 mo (n = 11)	29.9 \pm 5.4	27.2 \pm 5.1	1.8 \pm 0.8 [4]	16.2 \pm 3.2	9
2 mo (n = 13)	33.9 \pm 5.6	25.2 \pm 3.5	2.2 \pm 0.7 [2]	15.5 \pm 2.3	11
4 mo (n = 15)	31.4 \pm 4.4	25.0 \pm 5.0	1.9 \pm 0.7 [5]	16.7 \pm 2.9	10
8 mo (n = 13)	33.4 \pm 4.0	22.7 \pm 4.0	2.4 \pm 1.5 [4]	15.6 \pm 2.2	4
All (n = 60)	32.0 \pm 4.7	25.2 \pm 4.5	2.1 \pm 1.0 [17]	15.8 \pm 2.6	41
Nonlactating postpartum					
1–2 wk (n = 8)	29.7 \pm 6.9	28.4 \pm 6.6	1.4 \pm 0.5 [5]	13.5 \pm 3.2 ⁴	5
1 mo (n = 9)	28.1 \pm 6.4	28.5 \pm 5.6	1.6 \pm 0.7 [5]	13.2 \pm 1.9 ^{4,5}	8
2 mo (n = 6)	26.5 \pm 3.9	28.1 \pm 5.9	1.5 \pm 0.8 [4]	13.8 \pm 2.7	2
4 mo (n = 9)	29.7 \pm 6.6	31.5 \pm 8.4	2.0 \pm 0.9 [3]	13.8 \pm 1.8 ⁵	4
8 mo (n = 9)	27.5 \pm 5.2	25.4 \pm 2.8	2.6 \pm 0.7 [0]	12.9 \pm 3.0 ^{4,5}	3
All (n = 41)	28.4 \pm 5.8 ⁵	28.4 \pm 6.2 ⁵	1.8 \pm 0.8 [17]	13.4 \pm 2.4 ⁵	22
Postpartum (grouped by parity)					
Primiparous (n = 34)	28.4 \pm 5.8	25.1 \pm 4.8	1	15.1 \pm 2.7	23
Multiparous (n = 67)	31.6 \pm 5.0 ⁶	27.3 \pm 5.6	2.5 \pm 0.8	14.7 \pm 2.9	40

¹ $\bar{x} \pm$ SD.

²Number of women self-reporting multivitamin and mineral supplement use.

³Number of primiparous women in each group in brackets.

⁴Significantly different from nulliparous control subjects, $P < 0.05$.

⁵Significantly different from corresponding lactating group, $P < 0.05$.

⁶Significantly different from primiparous, $P < 0.05$.

Immunocytochemistry Systems, San Jose, CA); CD4-FITC/CD8-PE, markers for helper T and cytotoxic T cells, respectively (Dako Corp, Carpinteria, CA); and IgG1/IgG2a isotype controls (Olympus Immunochemicals, Lake Success, NY).

Flow cytometric staining and analysis

Monoclonal antibodies were added to 100 μ L whole blood and the mixture was incubated at room temperature for 15 min. Red blood cells were then lysed and leukocytes were fixed with a commercial reagent (AMAC Inc, Westbrook, MA). The cells were then washed twice in a 1% fetal bovine serum in phosphate-buffered saline solution, fixed in 1% paraformaldehyde in phosphate-buffered saline, and stored in the dark at 4–8°C for < 2 wk before analysis by flow cytometry (10).

The surface phenotypes of cells were examined with a FAC-Scan flow cytometer equipped with a 15-mW argon ion laser tuned to 488 nm (Becton Dickinson, Mountainview, CA). Appropriate isotype controls and electronic gating were used in each experiment. In analyses of two- and three-color data, an electronic gate was set on the lymphocyte population on the basis of forward- compared with side-angle light scatter as well as the percentage of cells within that gate staining for CD45. Quadrant markers in fluorescence dot plots were set by using matched, isotype-control stained samples.

For three-color analyses, a second electronic gate was set on cells staining CD3⁺ (PerCP) on the basis of the forward-scatter compared with fluorescence 3 (FL3) histogram. Data were analyzed with BD LYSIS II software (Becton Dickinson). Becton Dickinson CaliBRITE (Becton Dickinson Immunocytochemistry Systems) beads were run before each analysis to monitor instrument performance and to set the detector levels for forward- and side-angle light scatter as well as the fluorescence 1 (FL1), fluorescence 2 (FL2), and FL3 channels. For two- and three-color analyses, compensation settings (FL1 – %FL2 = \approx 1.0, FL2 – %FL1 = \approx 20, FL2 – %FL3 = 0.0, and FL3 – %FL2 = \approx 35) were optimized for each subject.

The immunofluorescence data on maternal T cells were analyzed statistically in two formats: as relative percentages (frequencies) and as absolute counts. Relative percentage data described what percentage of the gated cells (putatively lymphocytes) were phenotypically T cells. Being a proportional measure, the relative percentage was independent of changes in the concentration of lymphocytes or leukocytes in the blood. Absolute count data for each T cell subpopulation were obtained by multiplying the relative percentage of T cells by the concentration of lymphocytes in the blood sample. The lymphocyte concentration was calculated by multiplying the percentage of lymphocytes by the total white blood cell count. Thus, absolute counts of T cell subpopulations depended on the concentration of both lymphocytes and leukocytes.

Iron status analyses

Maternal iron status measures included hemoglobin concentration, mean cell volume, serum ferritin concentration, and serum transferrin saturation. The hemoglobin concentration and mean cell volume were measured with a Coulter STKS (Coulter Corp, Miami). Serum ferritin was measured by using a commercial microparticle enzyme immunoassay (IMx Ferritin, Abbott Laboratories, Abbott Park, IL). Serum transferrin saturation was calculated as follows: (serum iron/total-iron-binding capacity) \times 100. Serum iron and total-iron-binding capacity were analyzed

with a Kodak Ektachem E700 analyzer (Eastman Kodak Co, Rochester, NY). Monthly quality control tests confirmed that all iron assays maintained a CV < 5%.

Statistical analysis

To compare iron status, T cell numbers, and demographic data among the nulliparous, lactating, and NLPP groups, one-way analysis of variance (ANOVA) tests were performed (two-way ANOVA would exclude the control group). When a one-way ANOVA test had a significance of $P < 0.05$, Fisher's least-significant-difference method for multiple comparisons was used to determine the significance of individual between-group differences (11). To determine whether T cell numbers were correlated with iron status or other subject characteristics, multiple linear regression relations were tested with use of categorical variables for lactation status, multivitamin and mineral supplement use, or parity, and continuous variables for other maternal characteristics (eg, transferrin saturation, age, and time postpartum). Final results were based on the best fit of models derived from theorized relations (highlighted in the discussion) supplemented by information from forward stepwise regression tests ($P < 0.15$ to enter and remove) that included all variables from Tables 1 and 2. Simple linear regression was used to determine correlations between two variables. All regression models were tested for outliers, leverage points, nonlinear relations, and interaction terms. Statistical analyses were carried out with SYSTAT 5.2.1 for the Macintosh (SYSTAT Inc, Evanston, IL).

RESULTS

Health, demographics, and iron status

Of the 121 women recruited, two lactating (1–2 wk and 1 mo postpartum) and one NLPP (1–2 wk postpartum) subject with high white cell counts and elevated ESRs (> 20 mm/h) were removed from the analyses. One lactating (1 mo postpartum) and one NLPP (4 mo postpartum) subject also were removed from the analysis because < 80% of gated cells (putatively lymphocytes) from these subjects were CD45⁺ (normal range: 90–100%). One nulliparous control and one NLPP (4 mo postpartum) subject also were excluded because the percentage of CD3⁺ cells was < 50% of total lymphocytes (normal range: 70–85%). The exclusion of these seven subjects resulted in a final sample size of 114. Sample sizes in Table 1 do not include excluded subjects.

Collectively, NLPP mothers were significantly younger, heavier, and less educated than the lactating mothers in this study ($P < 0.05$). Some individual groups of NLPP mothers also had significantly less education than the nulliparous control subjects or the corresponding lactating group (Table 1).

The study population had normal to below-normal iron status (Table 2). Six subjects were anemic (hemoglobin concentration < 120 g/L). Three of the six had iron deficiency anemia (hemoglobin < 120 g/L and ferritin < 10 μ g/L). Nine subjects had poor iron status according to two of the following criteria: transferrin saturation \leq 15%, serum ferritin \leq 10 μ g/L, or mean cell volume < 80 fL. These women included one lactating (1 mo postpartum), seven NLPP (one each at 1–2 wk, 1 mo, and 2 mo postpartum, plus two each at 4 and 8 mo postpartum), and one nulliparous subject. Exclusion of any or all of these subjects did not substantively alter the correlations presented here; therefore, they were retained in the analysis.

TABLE 2
Iron status measures¹

Group	Mean red cell volume <i>fL</i>	Serum ferritin $\mu\text{g/L}$	Transferrin saturation %	Hemoglobin <i>g/L</i>
Control	88.1 \pm 4.6	320 \pm 180 (270) ²	24.3 \pm 13.9	135 \pm 8
Lactating				
1–2 wk	89.4 \pm 2.8	350 \pm 180 (270)	24.2 \pm 15.5	141 \pm 13
1 mo	89.9 \pm 3.1	380 \pm 60 (310)	26.9 \pm 14.2	134 \pm 6
2 mo	89.2 \pm 4.1	540 \pm 350 (450)	31.3 \pm 8.8	136 \pm 10
4 mo	87.7 \pm 2.9	400 \pm 260 (370)	30.1 \pm 12.8	140 \pm 7
8 mo	89.1 \pm 2.7	520 \pm 260 (490)	33.5 \pm 6.5	140 \pm 5
All	88.9 \pm 3.2	450 \pm 280 (390)	29.7 \pm 11.7	138 \pm 8
Nonlactating postpartum				
1–2 wk	91.1 \pm 4.8	580 \pm 550 (380)	19.2 \pm 9.5	117 \pm 44
1 mo	88.0 \pm 4.3	360 \pm 350 (210)	22.3 \pm 12.2	132 \pm 11
2 mo	86.1 \pm 3.9	160 \pm 40 (180)	16.9 \pm 7.5 ³	128 \pm 9
4 mo	88.5 \pm 4.6	380 \pm 520 (220)	21.6 \pm 8.1	135 \pm 7
8 mo	85.7 \pm 5.7	200 \pm 140 (190)	18.5 \pm 10.9 ^{3,4}	133 \pm 12
All	87.9 \pm 4.9	350 \pm 400 (200)	19.9 \pm 9.6 ³	132 \pm 10 ³
Postpartum (grouped by parity)				
Primiparous	88.4 \pm 4.3	350 \pm 350 (200)	24.6 \pm 14.2	135 \pm 11
Multiparous	88.6 \pm 3.8	440 \pm 320 (350)	26.3 \pm 10.5	136 \pm 9

¹ $\bar{x} \pm \text{SD}$.² Median in parentheses.³ Significantly different from corresponding lactating group, $P < 0.05$.⁴ Significantly different from nulliparous control subjects, $P < 0.05$.

By one-way ANOVA, the NLPP subjects had significantly lower transferrin saturation and hemoglobin concentrations than the lactating subjects ($P < 0.001$). The ranges of transferrin saturation percentages and hemoglobin concentrations for nulliparous control subjects were intermediate to those of the other groups (Table 2). By simple linear regression, maternal age was positively correlated with ferritin ($r = 0.36$; coefficient: 28.03), transferrin saturation ($r = 0.14$; coefficient: 25.98), hemoglobin ($r = 0.08$; coefficient: 1.53), and mean cell volume ($r = 0.09$; coefficient = 0.40) among all postpartum subjects ($P < 0.01$ for all correlations) but not with number of months postpartum.

Parity and iron status

The percentages and counts of CD3⁺ and CD3⁺CD8⁺ cells were not statistically related to time postpartum, even when maternal characteristics or iron status was included in the model (Table 3). Also, there were no significant differences in percentages or counts of CD3⁺ or CD3⁺CD8⁺ cells between the lactating and NLPP women (Table 3). The major contributors to variation in CD3⁺CD8⁺ cell percentages and counts were maternal parity (primiparous compared with multiparous), age, and percentage serum transferrin saturation.

Because there were no significant linear relations between parity and immunologic outcomes, postpartum women were grouped as primiparous or multiparous (parity of 2–6). The parity groups included approximately equal numbers of lactating and NLPP women with relatively equal representation among the five postpartum time points (Table 1). Primiparous, multiparous, and nulliparous groups did not differ significantly in any of the characteristics listed in Table 1, except that the primiparous group was significantly younger than the multiparous group ($P < 0.05$).

Women in the 1–2-wk and 1-mo postpartum groups were not included in the analysis of absolute counts of CD3⁺ or

CD3⁺CD8⁺ cells because leukocyte concentrations are likely to be changed to a variable degree by expanded plasma volume retained from pregnancy through the first 6 wk postpartum (12). Relative percentages of CD3⁺, CD3⁺CD4⁺, and CD3⁺CD8⁺ cells, however, are independent of changes in plasma volume (discussed above).

Helper T cell (CD3⁺CD4⁺) relative percentages and absolute counts did not differ significantly between the different parity groups (Table 3). When the multiparous and primiparous groups were combined, the postpartum women had significantly higher CD3⁺ relative percentages (77.2 \pm 6.0%, $P < 0.01$) and absolute counts (1460 \pm 405 $\times 10^3$ cells/L, $P < 0.05$) than did nulliparous control subjects. Both the multiparous and primiparous groups had significantly higher relative percentages ($P < 0.025$) and absolute counts ($P < 0.01$) of CD3⁺CD8⁺ cells than the nulliparous control subjects. Additionally, the primiparous group had significantly higher absolute counts of CD3⁺CD8⁺ cells than the multiparous group ($P < 0.025$).

With use of multiple linear regression, the relative percentages of CD3⁺CD8⁺ cells among the postpartum women were found to be correlated ($n = 101$; $P = 0.001$; $r = 0.42$; SEE = 6.01) with maternal age and transferrin saturation, the correlation with transferrin saturation being dependent on an interaction with maternal parity (Table 4). The same correlations were found when absolute counts of CD3⁺CD8⁺ cells were modeled ($n = 101$; $P = 0.001$; $r = 0.43$; SEE = 174). Thus, among multiparous women, the relative percentages and absolute counts of CD3⁺CD8⁺ cells were higher with increasing transferrin saturation (Table 4). Although the range of transferrin saturation percentages was similar among the different parity groups (Table 2), no positive correlation was found among primiparous or nulliparous women. Statistical models using one or more other measures of iron status (ferritin, mean cell volume, or hemoglobin) were not correlated with T cell percentages or counts.

TABLE 3
Percentages and absolute counts of blood T cells¹

Group	CD3 ⁺	CD3 ⁺	CD3 ⁺ CD4 ⁺	CD3 ⁺ CD4 ⁺	CD3 ⁺ CD8 ⁺	CD3 ⁺ CD8 ⁺
	%	×10 ³ cells/L	%	×10 ³ cells/L	%	×10 ³ cells/L
Control	72.4 ± 5.1	1198 ± 450	46.2 ± 7.2	768 ± 324	21.9 ± 4.9	361 ± 144
Lactating						
1–2 wk	75.3 ± 5.5	1297 ± 283 ²	44.7 ± 3.5	768 ± 158 ²	23.9 ± 5.1	417 ± 147 ²
1 mo	80.4 ± 5.4	1658 ± 481 ²	49.3 ± 8.7	1029 ± 316 ²	25.4 ± 8.3	523 ± 241 ²
2 mo	76.7 ± 7.2	1316 ± 207	42.1 ± 7.4	718 ± 133	27.1 ± 4.8	471 ± 134
4 mo	75.3 ± 4.5	1420 ± 338	42.5 ± 5.2	804 ± 227	26.6 ± 5.3	496 ± 133
8 mo	77.2 ± 5.5	1645 ± 513	43.1 ± 6.5	922 ± 342	28.0 ± 4.9	592 ± 206
All	76.9 ± 5.7	1475 ± 403	44.0 ± 6.7	847 ± 268	26.4 ± 6.3	507 ± 178
Nonlactating postpartum						
1–2 wk	74.8 ± 7.3	1221 ± 405 ²	40.9 ± 5.8	662 ± 213 ²	26.3 ± 3.0	431 ± 156 ²
1 mo	80.1 ± 5.4	1542 ± 419 ²	48.4 ± 8.5	911 ± 214 ²	25.1 ± 8.8	508 ± 301 ²
2 mo	79.7 ± 9.0	1702 ± 327	40.6 ± 3.5	871 ± 176	31.6 ± 5.4	671 ± 140
4 mo	76.3 ± 6.3	1322 ± 382	42.2 ± 7.5	728 ± 269	27.8 ± 8.0	474 ± 152
8 mo	77.0 ± 5.4	1442 ± 428	46.0 ± 5.3	863 ± 286	25.8 ± 6.5	486 ± 190
All	77.5 ± 6.6	1438 ± 411	43.9 ± 6.9	810 ± 245	27.0 ± 6.8	506 ± 208
Postpartum (grouped by parity)						
Primiparous	78.2 ± 5.9	1569 ± 443	43.8 ± 5.0	882 ± 279	27.3 ± 5.4 ³	546 ± 179 ⁴
Multiparous	76.7 ± 6.1	1409 ± 378	44.1 ± 7.5	808 ± 246	26.3 ± 7.0 ³	488 ± 193 ³

¹ $\bar{x} \pm \text{SD}$.² Data from this group were not included in the ANOVA test (*see* text).³ Significantly different from nulliparous control subjects, $P < 0.05$.⁴ Significantly different from nulliparous and multiparous groups, $P < 0.05$.

Parity, supplement use, and lactation status

Similar to the CD3⁺CD8⁺ data, the subset of variables predictive of CD3⁺CD4⁺ cell percentages was dependent on maternal parity. The percentages and counts of CD3⁺CD4⁺ lymphocytes did not change significantly over time nor were there significant differences between lactating and NLPP subjects when parity was excluded from the model. For lactating, primiparous women ($n = 17$), the only variable significantly associated with relative percentages (not absolute counts) of CD3⁺CD4⁺ cells was maternal age (mean age of this subset: 30.1 ± 5.1 y; regression equation: CD3⁺CD4⁺ percentage = 0.722(maternal age) + 22.264; $r = 0.73$, $P = 0.002$). None of the maternal demographic, iron status, or other variables under study were correlated with CD3⁺CD4⁺ percentages or counts in primiparous, NLPP mothers.

Among multiparous women, postpartum changes in percentages (not counts) of CD3⁺CD4⁺ cells were associated with an interaction between infant feeding (lactating compared with NLPP women) and maternal use of multivitamin and mineral supplements in addition to the independent effects of transferrin saturation and maternal age (Table 5). The equation for NLPP women who were not using multivitamin and mineral supplements at the time of the study had a significantly lower intercept and a more positive slope for the relation between time postpartum and CD3⁺CD4⁺ percentages than did the equation for NLPP women who were using supplements. In other words, NLPP women who were not using supplements had significantly lower CD3⁺CD4⁺ percentages around the first month postpartum than did NLPP women who were using supplements. NLPP women not using supplements had higher CD3⁺CD4⁺ percentages ≈4 and 8 mo postpartum than did NLPP women not using supplements at ≈1 mo postpartum but similar percentages to all NLPP women using supplements. This multiple linear regression model indicates that, at ≈8 mo postpartum, CD3⁺CD4⁺ percentages for NLPP women not using supplements were in the same range as those for the nulliparous control subjects (Table 2).

For lactating women, the equation for those who used supplements had a significantly more negative slope for the relation between time postpartum and CD3⁺CD4⁺ percentages than did the equation for lactating women not using supplements. In other words, lactating women who used supplements had incrementally lower CD3⁺CD4⁺ percentages over the first 8 mo postpartum whereas lactating women who did not use supplements did not show differences in CD3⁺CD4⁺ percentages over time. A three-way interaction between feeding, supplement use, and time postpartum was not significant ($P > 0.10$). After excluding women who were ≤1 mo postpartum (discussed above), absolute counts of CD3⁺CD4⁺ cells were not correlated with any subset of variables measured.

In multiparous, lactating women who used supplements, the decrease in CD3⁺CD4⁺ percentages was matched by an increase in CD3⁺CD8⁺ percentages over time (negative correlation by simple linear regression; $r = 0.52$, $P < 0.025$). Among multiparous NLPP women who were not using supplements, the increase in CD3⁺CD4⁺ percentages over time was not correlated with any change in CD3⁺CD8⁺ percentages over time.

DISCUSSION

This study was the first to compare maternal blood T cell subpopulations in lactating and nonlactating women postpartum. In this healthy population of women, maternal age and parity were the most significant predictors of T cell numbers, whereas lactation status and nutritional influences (eg, serum transferrin saturation and multivitamin and mineral use) were significant predictors only in multiparous women.

Our T cell results differed somewhat from those of previous studies in postpartum women. Gennaro et al (13) found that women 4 mo postpartum had higher percentages and counts of CD3⁺ cells than found in previous studies of healthy men and women. Maternal CD8⁺ cell percentages increased significantly over the first 2 mo postpartum followed by a decrease at 4 mo, but were otherwise

TABLE 4
Regression models for blood CD3⁺CD8⁺ cell relative percentages and absolute counts¹

Variable	Coefficient		SE		P	
	Percentage	Count	Percentage	Count	Percentage	Count
Constant	31.3	528	3.9	115	0.000	0.000
Primiparous versus multiparous ²	7.35	224	2.93	86	0.014	0.011
Transferrin saturation	0.287	8.69	0.076	2.21	0.000	0.000
Transferrin saturation × primiparous versus multiparous	-0.286	-6.89	0.104	3.02	0.007	0.025
Maternal age	-0.406	-8.84	0.129	3.78	0.002	0.022

¹ *n* = 101. For CD3⁺CD8⁺ percentages, *P* = 0.001, *r* = 0.42, and SEE = 6.01; for CD3⁺CD8⁺ counts, *P* = 0.001, *r* = 0.43, and SEE = 174.

² Categorical variable (primiparous = 1, higher parity = 0).

within the normal range. Iwatani et al (14) also found elevated CD3⁺ cell percentages at ≈4 mo postpartum but reported no postpartum difference in CD8⁺ cell percentages compared with nonpregnant control subjects. Other groups did not observe differences in CD3⁺ or CD8⁺ cell percentages or counts postpartum compared with those in nonpregnant control subjects (15–19). Although reduced CD4⁺ cell percentages during pregnancy is a frequent finding, some groups have reported significantly reduced CD4⁺ percentages and counts in the postpartum period (15, 16, 18), whereas other have not (14, 17, 19). One source of these differences may be our use of nulliparous control subjects whereas other groups used nonpregnant control subjects of unspecified parity.

The underlying immunologic picture is likely to be more complex than can be addressed with a descriptive study of total blood CD3⁺CD4⁺ and CD3⁺CD8⁺ cells. Pregnancy causes thymic involution in humans (20) and mice (21), a process that continues during lactation with a selective loss of immature (CD4⁻CD8⁻) T cells in the thymus (21). Iron deficiency is also associated with reduced thymus weight (22) and thymic protein synthesis (23) in rodent models. Poor iron status could interact with lactation to delay thymus regeneration after weaning and reduce immature T cell production. Parity may be another factor in this process because multiparous mice differ from age-matched, virgin female mice in the ratio of memory-to-naïve splenic T cells (24). To better address these issues, future human studies should include phenotypic markers for memory and immature T cells.

Correlations with iron status

Both CD3⁺CD8⁺ and CD3⁺CD4⁺ cell percentages were correlated with maternal age and transferrin saturation in multiparous

women. The increase in CD3⁺CD4⁺ and decrease in CD3⁺CD8⁺ percentages with increasing age were reported in previous population studies (25, 26). The negative correlation between CD3⁺CD4⁺ percentages and transferrin saturation, however, does not agree with a previous report of reduced helper T cell percentages in adults during iron deficiency (7) and is likely a statistical anomaly resulting from the correlation between CD3⁺CD8⁺ cell percentages and transferrin saturation. CD3⁺CD4⁺ and CD3⁺CD8⁺ cells comprise virtually all (97–99%) of the CD3⁺ cells found in peripheral blood; thus, measures of CD3⁺CD4⁺ and CD3⁺CD8⁺ percentages are highly correlated (*r*² = 0.783, *P* < 0.001). Unlike the findings for CD3⁺CD4⁺ cell percentages, transferrin saturation was correlated with both percentages and counts of CD3⁺CD8⁺ cells. On the basis of the data presented and biological plausibility, the nonspurious association is likely between transferrin saturation and CD3⁺CD8⁺ cells.

This study found a linear correlation between markers of immune status and iron status in adult humans. In this case, transferrin saturation was more predictive of T cell numbers than earlier (eg, ferritin) or later (eg, hemoglobin) markers of iron deficiency. Because serum transferrin-bound iron is the primary source of iron for lymphocytes (8), one can speculate that T cell numbers respond to available circulating iron rather than to iron stores when a person is not severely iron deficient (as found in this population). Animal studies have also shown that the degree of iron deficiency influences the degree of immunosuppression (27, 28). The implication of these graded outcomes is that iron-related changes in immune status in humans begin before iron stores are fully depleted.

Rather than poor iron status leading to a reduction in CD3⁺CD8⁺ cell percentages, an alternative hypothesis is that

TABLE 5
Regression models for relative percentages of blood CD3⁺CD4⁺ cells in the multiparous subjects¹

Variable	Coefficient	SE	P
Constant	22.50	6.81	0.002
Feeding status ²	13.24	4.36	0.004
Supplement use ³	14.81	4.20	0.001
Feeding status × supplement use	-10.43	3.92	0.010
Months postpartum	1.67	0.59	0.007
Months postpartum × feeding status	-1.82	0.68	0.010
Months postpartum × supplement use	-1.70	0.67	0.014
Transferrin saturation	-0.27	0.09	0.004
Maternal age	0.52	0.20	0.011

¹ *n* = 67, *r* = 0.60, and SEE = 6.5 for overall model.

² Categorical variable (lactating = 1, nonlactating postpartum = 0).

³ Categorical variable (taking multivitamin and mineral supplements = 1, not taking supplements = 0).

CD3⁺CD8⁺ cells have a role in regulating iron uptake. de Sousa et al (29) proposed that CD8⁺ lymphocytes at the intestinal epithelium basolateral surface can regulate iron uptake. Patients with hereditary hemochromatosis (characterized by abnormal iron uptake) often have reduced percentages of CD8⁺ cells (30) or defective CD8⁺ cells (31). This hypothesis predicts that an individual's iron status is positively correlated with CD8⁺ cell percentages or counts as iron absorption is increased. The relevance of this theory to normal or poor iron status remains to be shown.


Correlations with supplement use

NLPP and lactating women included in the study differed in several demographic variables that may have been a source of the observed differences among primiparous, lactating, and NLPP mothers. In the United States, these demographic factors are associated with the decision not to breast-feed and, given the available study population, could not realistically be removed as confounding factors.

Unlike the CD3⁺CD8⁺ cell data, the findings for percentages of CD3⁺CD4⁺ cells could not be extended to counts of these cells. For multiparous NLPP women, the greatest difference in percentages of CD3⁺CD4⁺ cells was seen in the first month postpartum. Women in the first month postpartum, however, were excluded from the analysis of absolute cell counts (as described above), making this difference undetectable. For multiparous lactating women, the exclusion of women at the first two time points reduced the sample size and increased the SE of the slope enough to make the effect of supplement use nonsignificant. To demonstrate the effects of postpartum supplement use on absolute counts (and not just relative percentages) of CD3⁺CD4⁺ cells would require either a larger sample size or a sampling method that takes into account the degree of hemodilution in each subject during the early postpartum period.

This study suggests that multiparous women who do not breast-feed their infants nor take multivitamin and mineral supplements have a significant deficit in CD3⁺CD4⁺ cell percentages in the early postpartum period. Because the effects were seen at the earliest time points examined, it is likely that the immunologic deficit resulted from practices during or before pregnancy that may have included prior avoidance of prenatal supplements or other forms of prenatal care. If so, these findings support the well-documented need for adequate prenatal care to promote maternal health.

The finding more difficult to explain was that lactating women who were using supplements had significantly decreased CD3⁺CD4⁺ cell percentages between 2 and 8 mo of lactation. The effect was not due to lactation alone, because lactating women who were not using supplements did not show decreases in CD3⁺CD4⁺ cell percentages.

If better iron status is associated with increased numbers of CD3⁺CD8⁺ cells in multiparous women, this effect could give these women an advantage in responding to intracellular infections and augment immunologic surveillance for precancerous cells (32–36), if the additional CD3⁺CD8⁺ cells are fully functional. Given the limited range of iron status in this study, one cannot predict the benefit or detriment of this relation between iron and immune status for women in economically developing countries, where the prevalence of concurrent iron deficiency, infection, and breast-feeding would be highest and the potential public health importance would be greatest. 

We gratefully acknowledge the assistance of the staff at Bassett Healthcare in Cooperstown, NY, and at the ARS US Department of Agriculture Children's Nutrition Research Center in Houston. The technical assistance of Trent Ihne and Sarah Pryputniewicz is also appreciated. Additional technical advice and assistance provided by Estelle Goodell (Bassett), Roger Davidson (Bassett), Kim Palkowetz (UTMB), and Linda Bennett (Cornell) were invaluable at all stages of this project.

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