Immunity, Inflammation and Infection in Post-partum Breast and Formula Feeders

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Problem
Little is known about immunological recovery in post-partum women and if lactational status affects immunocompetence. Many physiological changes occur, such as uterine involution and recovery of non-pregnant immune status. These changes may also affect susceptibility to disease.

Method
The study compared immune and inflammatory activation markers (serum cytokines, Epstein Barr viral antibody titer, neopterin, c-reactive protein (CRP), lymphocyte subset percentages, *ex vivo* cytokine production, lymphocyte proliferation, salivary Immunoglubulin A (sIgA) in exclusively breastfeeding (EB) and exclusively formula feeding (EF) women at 4–6 weeks post-partum, and control (C) women.

Results
EBs had higher ratios of interferon-γ (IFN-γ)/interleukin-10 (IL-10) than EFs, and post-partums were higher than Cs. Both IFN-γ and IL-10 were significantly higher in post-partums compared to controls. Post-partums also had higher proinflammatory cytokines, CRP and neopterin, salivary IgA and fewer infection symptoms. There were also differences in lymphocyte subsets.

Conclusions
The post-partum period is oriented towards heightened and activated innate and specific immune defenses, with breastfeeding providing a boost in these phenomena.

Introduction

The post-partum period has been thought of as a time for immunological 'recovery,' lasting about 1 year,[1] from the profound immunological changes of pregnancy. The differential rates of change in various aspects of immune function have not been extensively investigated. The role of lactational status in human immune studies of the post-partum has hardly been considered. In the animal world all post-natal mothers are lactating, but this is not the case for human mothers. Many patterns of feeding are present, including exclusive lactating, combined feeding, exclusive formula feeding, pumping, on demand and scheduled feeding, and co-sleeping. Consideration of lactational status in the immunology of the post-partum should be a concern, as lactation is a unique neuroendocrine state, associated with low levels of estrogen and progesterone, and high levels of oxytocin and prolactin. These hormones are believed to affect the HPA axis, making it refractory to stress in lactating dams.[2,3] The altered stress axis and lactational hormones have the potential to influence post-natal maternal immunology. From a teleological perspective, it makes sense that lactating mothers would be immunologically protected from external

threats, and even illness, in order to provide maximum protection and nurturance for her offspring.

The early post-partum period has been associated with up-regulated inflammatory responses. Delivery is associated with increased serum levels of inflammatory cytokines such as interleukin-6 (IL-6) and IL-1.[4,5] A broad state of immune activation is also characteristic of the early post-partum, as measured by levels of neopterin, soluble IL-2 receptor, and soluble CD8 antigen.[6] This may help the woman recover from the biological stress of birth, but little is known about the magnitude and length of such a state. Immune and inflammatory activation in post-partum women may be factors in anxiety and depression in the early days post-delivery.[7] CD4 cell counts are reported to rise in the post-partum, primarily accounted for by $\gamma\delta$ T cells.[8] Natural killer (NK) subsets with weak cytotoxic activity ($CD16^+$ $CD57^+$) were found to increase after delivery during months 1–4 post-partum.[1] Lymphocyte proliferation with phytohemagglutinin (PHA) was higher than non-post-partum controls in post-partum breastfeeders.[9]

To understand and frame post-partal immune recovery, consideration must be given to immunity and inflammation during pregnancy. The unique and enigmatic immune processes of the normal human pregnancy remain poorly understood and previous conceptualizations of T helper 1 (Th1) suppression and Th2 up-regulation during pregnancy are simplistic. The recognition of the critical role of T regulatory cells ($CD4^+$ $CD25^{+high}$) in suppressing fetal rejection in human pregnancy[10] and the mediating and protective role of the innate immune system during pregnancy[11] has provided new insights. Thus, the immunology of the post-partum cannot be viewed as merely a restoration of Th1/Th2 balance. This paradigm has been useful, but is not the only explanatory model, in explaining rheumatoid arthritis (RA) and autoimmune thyroid disease as Th1-driven diseases which remit during pregnancy but exacerbate during the post-partum.[12] One of the most common times for an initial diagnosis of RA is during the post-partum period, with breastfeeding increasing the risk.[13] Some research supports that lymphocytes from breastfeeding mothers produce more Th1 cytokines in *ex vivo* cultures compared to formula feeders and controls.[14,15] Both Th1 and Th2 *ex vivo* cytokine production rose during the post-partum compared to pregnancy, with increased Th1/Th2 ratios observed through post-partum months 1–12. Interferon-γ (IFN-γ) levels in culture supernatant of whole blood stimulated with phorbol 12-myristate 13-acetate and ionomycin were correlated with serum prolactin in breastfeeding mothers in these studies. While the behavior of blood mononuclear cells in culture under appropriate mitogenic simulation can be shown to polarize towards Th1 or Th2 cytokine production, the immunobiology of the whole organism is vastly more complex. Signaling of cytokine production by a multitude of cells involves 'interpretation' of danger by the host or tolerization when danger is not perceived.[16] The first defense is the innate immune system, an ancient and evolutionarily conserved response to infectious microbes. It consists of cells such as dendritic cells, macrophages, and endothelium, which have on their surfaces toll-like receptors (TLRs), which recognize molecular patterns associated with microbes (PAMPS). This recognition initiates a cascade of responses, both innate and ultimately specific if necessary, to destroy the microorganisms or infected cells. Regulation of this inflammogenic process is achieved in several ways. Cortisol secretion through stress responses down-regulates inflammation, as do cytokines such as IL-10 and transforming growth factor-β (TGF-β). Th2 lymphocytes secrete anti-inflammatory cytokines such as IL-10 as do T regulatory cells (Treg cells). Treg cells, representing 2–5% of $CD4^+$ cells, are believed to be important in immune tolerance by producing anergy and suppression of autoreactive T-cell clones.[17] They also appear to play a major role in pregnancy, increasing early, peaking in the second trimester and then declining in the post-partum.[18] T regulatory cells appear to be immunoregulatory during pregnancy and act to inhibit the immunostimulation of T cells through cell–cell contact or release of cytokines such as IL-10 and TGF-β.[24] T regulatory cells express TLRs on their surface, and danger signals such as LPS can cause these cells to proliferate and secrete cytokines. However the concentration of LPS that elicits this anti-inflammatory effect is much greater than that which activates TLRs on dendritic cells and macrophages. Thus it would appear that the T regulatory response is provoked when large amounts of LPS are being produced such as in septicemia, to control excessive inflammation produce by cytokines such as IL-6 and tumor necrosis factor-α (TNF-α).[19] During pregnancy the innate immune system appears activated but the post-partal innate immune system has not been well described, and the role of lactational status on

both innate and specific immune processes is unknown.

We report here a comprehensive comparison of inflammatory, immune, endocrine, and infectious illness differences in 181 exclusively breastfeeding (EBs) and exclusively formula feeding (EFs) women measured cross sectionally at 4–6 weeks post-partum, compared to age matched Cs.

Methods

The study was approved by the appropriate IRBs and women were recruited in the post-partum unit of the university hospital in a southern US city. While 300 mothers were recruited and agreed to participate, we retained 181 out of this original pool. The major reason that a participant was not retained is that she began to supplement and was therefore no longer an EB. Mothers were required to be either EBs or EFs from birth, and when a home visit revealed that an EB was supplementing, she was dropped from the study. Exclusion criteria were ages under 18 or over 45-year old, serious complications during pregnancy, labor, and delivery, chronic mental or physical illnesses, and medications that are known to influence immune function. Thirty-three control subjects were undergraduate and graduate female student volunteers who completed the study instruments and provided a morning blood sample in the lab. Participation took place during an academic exam free period. Exclusion criteria were current or chronic illness or pregnancy. All participants received a monetary gift for their time.

Mothers were visited in their homes by a research nurse between the morning hours of 8:00–11:00 AM. They were instructed to feed the baby as usual before the visit. They collected a 5-minute saliva specimen by the drool method, observing no eating, smoking, or tooth brushing for 1 hr before collection. A venipuncture was performed and 10 mL of blood was collected into a serum separator tube and into sterile heparinized vacutainers. Milk samples were also collected but that data are not reported in this paper. The serum tube and saliva were placed on ice and the samples were immediately transported to the lab. The serum was spun at 3800 *g* for 25 min, aliquoted, and frozen at −20°C. The heparinized blood was diluted 1:5 with RPMI supplemented with glutamine and 50 μg/mL gentamycin, with no added fetal calf serum (FCS). One mL aliquots were added to wells to which 5 μg/mL *E. coli* lipopolysaccharide (LPS) (Sigma–Aldrich) and 5 μg/mL PHA (ICN Biomedicals) were added. The cultures were incubated for 66 hr as were the lymphocyte proliferation cultures. Time curves were done to determine optimal length of incubation and the cultures produced maximal cytokines at 36 hr, but no loss of cytokines was observed at 66 hr. The cultures were spun at 1500 *g* for 5 min and the supernatant was aliquoted and frozen at −80°C until analysis. The remainder of the heparinized blood was prepared for flow cytometry or lymphocyte proliferation. One hundred μLs of blood were pipetted into plastic capped tubes and incubated with CD3, CD4, CD8, CD19 and CD56 antibodies as well as antibody controls and blanks. The antibodies (BD Biosciences) were either FITC or PE conjugated. The cells were prepared using Immunolyse kits (Beckman Coulter), and fixed for analysis in a Beckman Coulter Epics XL flow cytometer which can analyze up to four fluorochromes with a single argon air-cooled laser. For lymphocyte proliferation studies, the heparinized blood was pipetted onto 4 mL of Histopaque-1077 (Sigma–Aldrich) in 12 mL conical tubes, and the Ficoll preparation was then spun at 22°C at 1200 *g* for 30 min. The mononuclear cell layer was removed and washed in RPMI-1640 (Fisher) with 10% FCS, 50 μg/mL gentamycin, and glutamine at 1200 *g* for 10 min, then resuspended and counted by Trypan blue exclusion using a hemocytometer. Cells were suspended in RPMI and pipetted into wells in a 96 well plate at a concentration of 1,000,000 cells per well. Media and mitogens were added for a final volume of 200 μLs per well. Controls and stimulated assays were done in triplicate. PHA was added to one set of wells in two concentrations (1 and 5 μg/mL). Concanavalin A (ConA) was added at 0.1, 1, 5, and 10 μg/mL to wells. The plates were incubated in a 37°C incubator with 5% CO_2 for 66 hr. At that point 0.5 μCi of tritiated thymidine (ICN Biomedicals) was added to each well and the cultures were incubated an additional 6 hr. The plates were then harvested in a cell harvestor (Filtermate Harvester, Packard) onto glass fiber filter paper, cut into discs and added to liquid scintillation media and counted in a Tricarb 2100 TR scintillation counter (Packard). Background counts per minute (c.p.m.) were subtracted and the data was expressed as stimulation index (SI) (stimulated/control).

All cytokines (IL-10, IL-6, TNF-α, IFN-γ, and IL-2) were measured using kits from eBioscience. Prolactin and Epstein Barr viral (EBV) VCA antibody (Hope Labs), c-reactive protein (CRP), ACTH and cortisol (DRG), and neopterin (ALPCO) were measured by ELISA. The plates were read at the appropriate wavelengths on a Scantron plate reader and the data were further analyzed using the Prism GraphPad program. The inter- and intra-assay coefficients of variation were all less than 10%.

Demographic data were collected by a questionnaire and symptoms of infection collected through the Carr Infection Symptom Checklist (Carr SCL). The Carr SCL, developed by the investigators, consists of 30 symptoms, ranked according to severity on a 0–4 points Likert scale. There are respiratory, gastrointestinal, genitourinary, skin/eye, and general flu subscales. It has been pilot tested on over 400 subjects across all seasons. A factor analysis found a five-factor solution accounted for 48% of variance. Factor loadings indicated high loadings of appropriate symptoms on respiratory, genitourinary, and gastrointestinal subscales. Cronbach's alpha for the respiratory subscale was 0.87, for skin/eye, 0.397, for gu, 0.43, for gi, 0.55, and for flu, 0.45. Since respiratory symptoms are less disparate than other symptoms, these reliabilities make sense. Means of subscales are total, 7.43 ± 0.6, respiratory, 3.31 ± 0.35, skin/eye, 1.24 ± 0.145, genitourinary, 0.729 ± 0.117, gastrointestinal, 1.67 ± 0.13, general flu, 0.66 ± 0.10.

Results

Demographic Differences

Data were collected on 33 Cs, and 181 mothers of whom 85 were EBs and 96 were EFs. Since US women who breastfeed tend to be socioeconomically privileged, we made efforts to recruit as equivalent a sample of EBs and EFs as possible. For the post-partum group, mean age of EBs was 25.7 years, parity 1.87, income 25,000,64% were married and 22% worked full–time. The EFs mean age was 23.3 years, parity was 1.84, income was 12,000, 55% were married, and 17% worked full-time. Racial distribution of the entire sample was 84% Caucasian, 7.5% Black, 5% Hispanic, and 1.7% Asian. Mean time of data collection was 5.1 weeks post-partum. The Cs were all Caucasian and the mean age was 23.8 years. Statistically significant demographic differences between EBs and EFs were as follows: breastfeeders were older, had higher income, fewer smoked, and more were married. There were no differences in weeks post-partum, type of delivery, labor length, number of minor delivery complications, number of physician visits, current BMI, number of children, exercise patterns, race, and work status. Cs differed significantly from post-partum women in that they had lower body mass indices, and fewer were married.

Differences between Breast and Formula Feeders

Exclusively breastfeeders and EFs differed in several ways. EBs had lower CD3 and CD4 percentages. CD3 percentage in EBs was 75.9% compared to 79.4% in EFs ($t = 1.3$, $P = 0.036$), and the CD4 percentage was also lower in EBs (42.4 compared to 46.8%), but this was only marginally significant ($P = 0.09$).

Interferon-γ and IL-2 were both higher in EBs, but only IFN-γ was significantly different ($t = 2.09$, $P = 0.039$). The proinflammatory cytokines TNF-α and IL-6 were also higher in EBs but not significantly. Serum levels of IL-10 were not different. Examination of the production of *ex vivo* cytokines IFN-γ, IL-10, IL-6, and TNF-α in the supernatants from LPS and PHA stimulated whole blood cultures uncovered a significantly higher IL-10 production in EFs ($t = 2.2$, $P = 0.026$). EBV capsid antigen Ig titers were lower in breastfeeders (1.3 u/mL) compared to formula feeders (1.56 u/mL) ($t = 1.9$, $P = 0.05$). Neopterin and CRP levels were not different between EBs and EFs. Salivary IgA concentrations were not different between EBs and EFs, and were not correlated with milk IgA concentration in EBs.

Lymphocyte proliferation in PHA and ConA stimulated cultures differed. The SI was achieved by dividing mean stimulated c.p.m. by control culture mean c.p.m. The SI for EBs at a concentration of 5 μG/mL PHA was 219% compared to 136% for EFs ($t = 2.14$, $P = 0.036$). The lower concentration of 1 μG/mL produced equivalent stimulation, and all concentrations of ConA (0.1, 1, and 5 μG/mL) produced greater stimulation in the EB's cultures but none of these values reached statistical significance.

With regard to symptoms of infection, there were differences in both severity and frequency scores between EBs and EFs. Severity scores were significantly lower for respiratory ($t = 2.6$, $P = 0.01$) and skin and eye ($t = 2.35$, $P = 0.01$) subscales, and total infection symptom score ($t = 2.1$, $P = 0.04$) in the

breastfeeders. Frequencies of various types of symptoms showed that breastfeeders had less total frequency of infection symptom reports except for genitourinary symptoms, which were higher in breastfeeders.

Correlates of Immune, Endocrine, and Infection Variables in Post-partum Mothers

Since we collected data from mothers over the fourth through sixth week post-partum, we determined if the length of time since delivery influenced immune parameters. The only variable affected by the time since birth was serum IL-2, which was correlated with weeks post-partum ($r = 0.2$, $P = 0.016$) and mitogen proliferation, which was inversely correlated with weeks post-partum for both ConA ($r = -0.22$, $P = 0.008$) and PHA ($r = -0.18$, $P = 0.05$). Other demographics were examined as well. BMI was negatively correlated with the serum IFN-γ/IL10 ratio ($r = -0.18$, $P = 0.04$), and smoking was associated with higher serum IFN-γ ($r = 0.163$, $P = 0.049$).

Symptom severity scores were correlated with CD3 ($r = 0.36$, $P = 0.015$), CD4 ($r = 0.46$, $P = 0.001$), and CD19 percentages ($r = 0.378$, $P = 0.015$), but only in EBs. Lymphocyte subsets were not correlated with infection in EFs at all. Genitourinary ($r = -0.49$, $P = 0.006$), gastrointestinal ($r = -0.326$, $P = 0.049$), and flu symptom ($r = -0.39$, $P = 0.03$) subscales were negatively correlated with whole blood stimulated culture production of IL-6, but again only in EBs.

Serum prolactin was correlated with PHA stimulated lymphocyte proliferation ($r = 0.23$, $P = 0.016$) but not significantly with ConA stimulation ($r = 0.13$, $P = 0.12$). Serum prolactin was correlated with serum IL-2 ($r = 0.24$, $P = 0.046$). The expected suppression of immune function by ACTH and cortisol was not observed in the post-partum mothers. None of the serum cytokines or *ex vivo* cytokine production (IFN-γ, IL-10, IL-6, and TNF-α) was correlated with either ACTH or cortisol in these mothers. The only immune parameter apparently related to these stress hormones was lymphocyte proliferation. PHA ($r = -0.16$, $P = 0.08$) and ConA ($r = -0.12$, $P = 0.02$) stimulated lymphocyte proliferation seemed to be suppressed when levels of cortisol were high. In contrast, controls had correlations between cortisol and ACTH and several immune parameters. Cortisol was correlated inversely with *ex vivo* IFN-γ production ($r = -0.33$, $P = 0.05$) and serum IFN-γ ($r = -0.33$, $P = 0.05$) and ACTH was related to serum levels of IL-10 ($r = 0.40$, $P = 0.02$), IL-6 ($r = 0.26$, $P = 0.06$), TNF-α ($r = 0.5$, $P = 0.003$), CD8 percents ($r = 0.50$, $P = 0.003$) and CD56 percents ($r = 0.35$, $P = 0.05$) in Cs.

Many of the post-partum immune measures were highly intercorrelated, as would be expected since they are variables that reflect aspects of immune activation. For example IL2, IFN-γ, IL-6, CRP and TNF-α were all highly correlated with each other (see Table I).

Comparisons of Post-partum Women with Controls

Post-partum mothers as a group (EBs and EFs) were compared to the 33 control women. These data are presented in Tables II, III, and IV. To summarize, post-partum women appeared to have a generally up-regulated and heightened innate and specific immune system, and higher basal cortisol and ACTH levels compared to controls. Post-partum women had higher serum levels of IL-10, IFN-γ, IL-2, IL-6, TNF-α, neopterin, and CRP. The serum IFN-γ/IL10 ratio was higher for the post-partum group. The

Table I Pearson Correlation Coefficients for Serum Cytokines and Inflammatory Proteins

	CRP	Neopterin	IFN-γ	IL-2	IL-6	TNF-α
Neopterin	NS					
IFN-γ	NS	NS	–			
IL-2	$r = 0.56$***	NS	$r = 0.4$***			
IL-6	NS	$r = 0.32$***	$r = 0.35$***	$r = 0.36$***		
TNF-α	NS	$r = 0.2$**	$r = 0.5$***	$r = 0.27$***	$r = 0.4$***	
IL-10	$r = -0.37$***	$r = 0.15$*	$r = 0.39$**	$r = 0.18$*	$r = 0.36$***	$r = 0.5$***

CRP, c-reactive protein; IFN-γ, interferon-γ; IL, interleukin; TNF, tumor necrosis factor-α; NS, not significant.
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table II Serum/Plasma Variables in Post-partums Compared to Controls

	Cortisol (ng/mL)*	ACTH (pg/mL)**	CRP (mg/L)**	Neopterin (nmol/L)**	IL-6 (pg/mL)**	TNF-α (pg/mL)***	IFN-γ (pg/mL)***	IL-10 (pg/mL)*	EBV VCA titer (units)
Post-partums	167.57	7.96	8.6	7.43	4.8	4.2	11.39	6.76	1.67
Controls	118.53	2.72	3.7	6.2	1.9	0.85	5.88	2.60	1.32

EBV, Epstein Barr viral; CRP, c-reactive protein; IFN-γ, interferon-γ; IL, interleukin; TNF, tumor necrosis factor-α.
*$P < 0.01$ and **$P < 0.001$.

Table III Lymphocyte Percentages and Ratios in Post-partums Compared to Controls

	CD3	CD4	CD8**	CD19*	CD56**	CD4/CD8*
Post-partums	74.89	44.92	4.9	23.08	0.91	2.17
Controls	75.82	45.99	12.63	19.36	7.26	1.76

*$P < 0.01$ and **$P < 0.001$.

Table IV *Ex Vivo* Production of Cytokines in LPS and PHA Stimulated Cultures

	IFN-γ (ng/mL)	TNF-α (pg/mL)	IL-10 (pg/mL)*
Post-partums	260.4	3121.6081	2024.1443
Controls	276.2	3121.0625	2791.7455

IFN-γ, interferon-γ; IL, interleukin; TNF, tumor necrosis factor-α.
*$P < 0.01$.

lymphocyte proliferation with PHA was higher, and there were significant differences in lymphocyte subsets. The only significant *ex vivo* cytokine production difference was the higher levels of IL-10 produced in control cultures compared to post-partums. Salivary IgA was higher in post-partums (282 μg/mL) compared to controls (79.6 μg/mL). The original control group did not provide saliva, so saliva samples were collected from six female co-workers and analyzed for comparison. Salivary IgA was correlated with serum IL-6 ($r = 0.32$, $P < 0.001$).

Analyses of covariance using marital status and income as covariates were performed for the biological data and infection symptoms. These covariates were chosen, as these variables were significantly different between EBs and EFs. There were no significant contributions of these covariates to the variances in all but the flu and total SCL scores for marital status and for flu symptoms for income. Data for EBs, EFs, and controls are presented in Figs 1–5.

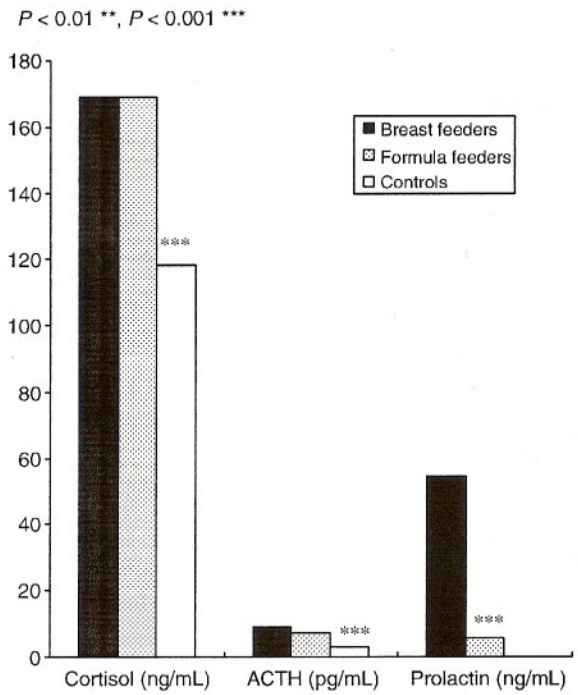

Fig. 1 Cortisol and ACTH are higher in post-partum women compared to controls. Prolactin not measured in controls.

Discussion

While there were differences between EFs and EBs, the more striking differences were between post-partum women and controls. These differences suggest

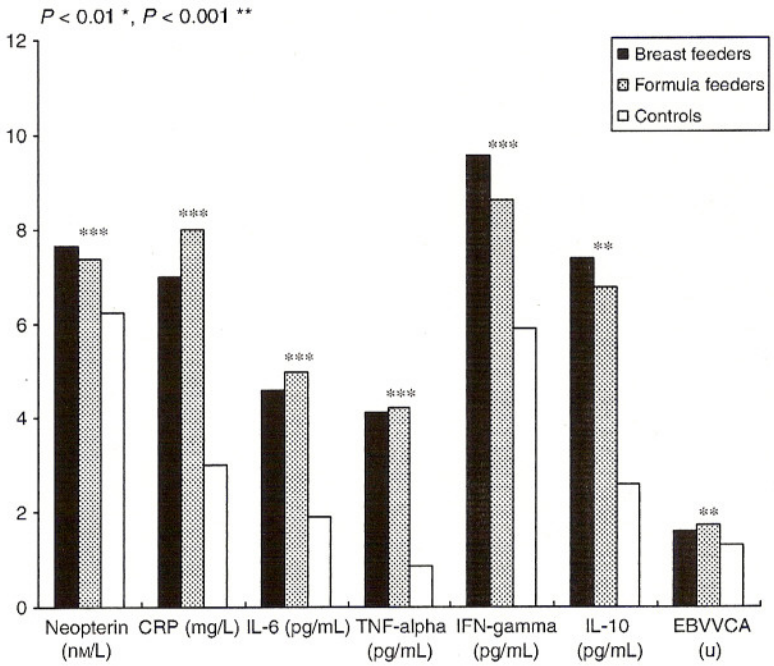

Fig. 2 Inflammatory markers and cytokines are higher in post-partum women compared to controls.

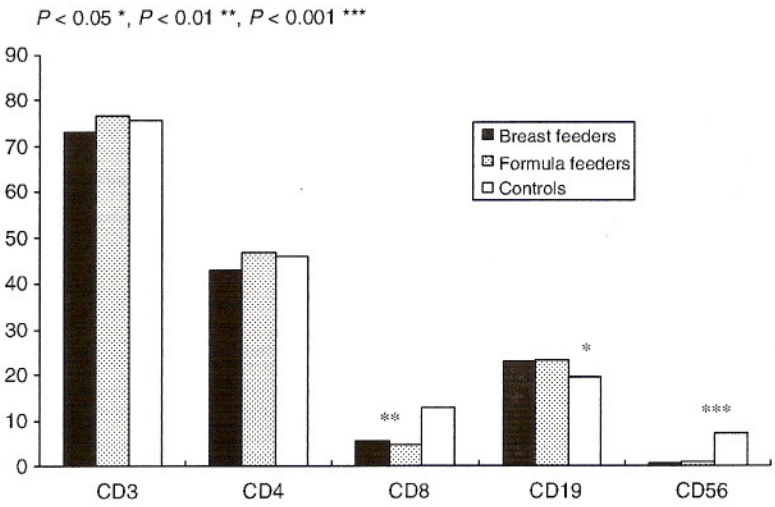

Fig. 3 Percentages of lymphocytes measured by flow cytometry indicate differences in CD8, CD56, CD19, and the CD4/CD8 ratio.

that post-partum women in general, irregardless of feeding status, have a more activated immune system and up-regulated innate inflammatory response. EBs appears to have a somewhat more enhanced cellular immune response (higher IFN-γ, Lower EBV VCA antibody titer), and to have higher serum levels of proinflammatory cytokines than EFs and they also report fewer symptoms of infection. These differences generally become magnified when EFs and EBs as a group are compared to control women. Perhaps most pertinent is the fact that every serum cytokine measured and all proinflammatory macrophage and acute phase proteins (TNF-α, IL-6, CRP, and neopterin) are higher in post-partum mothers. Along with a higher lymphocyte proliferative response, these data suggest a broad state of immune readiness and activation. Included in this activation state is the secretion of higher levels of secretory

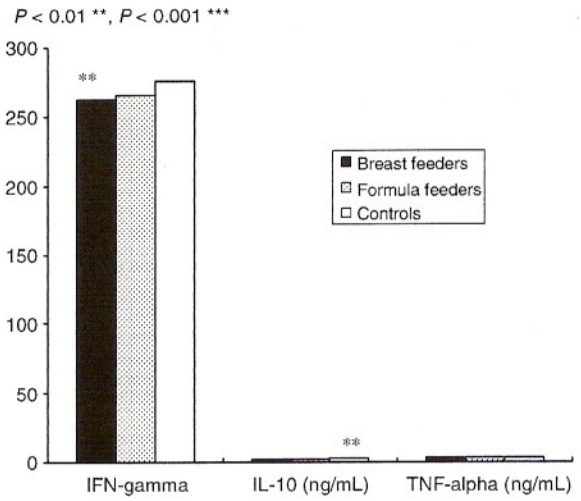

Fig. 4 *Ex vivo* cytokine production in LPS and PHA stimulated whole blood cultures.

IgA, suggesting up-regulated mucosal immunity. The role of this state and the activation signals, which are obviously not classical PAMPs, are unclear at this time. It is tempting to speculate that innate inflammatory activation is protective. But it is also important to acknowledge that the innate response may be related to the stage of uterine involution. There is increased risk for maternal uterine contamination during birth, and the endometrium is protected from infection by activated macrophages. Uterine involution takes about 4–6 weeks in humans, and is associated with shrinkage of the myometrium, elimination of microorganisms, and repair and restoration of the endometrium. A process of remodeling occurs which is associated with both apoptosis and proliferation. IL-2 activated NK cells ($CD56^{+bright}/CD16^{-}$) participate in this process through secretion of both matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs).[20] While the molecular biology of human involution is not well known, it seems apparent that this process involves inflammatory and immune mediators. Inflammation of the cervix has been reported in 92% of post-partum women at 4 weeks, and 88% at 6 weeks.[21]

The *ex vivo* cytokine data do not parallel the peripheral blood levels. In this whole blood assay, the cells are stimulated by LPS and PHA, and lymphocytes and NK cells release IFN-γ while stimulated monocytes release IL-10. Others have found increased type-1 cytokines in stimulated whole blood cultures from post-partum women.[14,15] They also found elevations in *ex vivo* IL-10 production. These cultures were stimulated by phorbol myristate acetate and ionomycin, but the concentrations of cytokines produced were far lower than our cultures so it is difficult to compare these data. The lack of strong relationships between

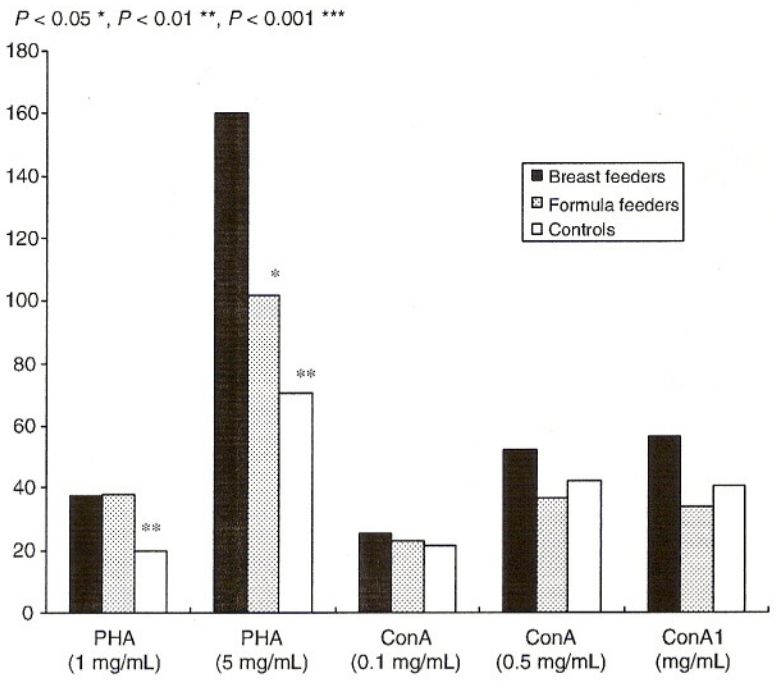

Fig. 5 Breastfeeders show greater proliferation stimulation index (SI) compared to formula feeders, and to controls in PHA stimulated cultures, but not with ConA stimulation.

the *ex vivo* data and peripheral blood may be explained by the complexity of the systemic and local immune responses in the whole organism. Many cells secrete inflammatory and proimmune cytokines, and the measures in peripheral blood are determined not only by cellular release of cytokines, but also uptake into tissues, and binding to receptors.

Some evidence for an increase in Th1 function in breast compared to formula feeders is present in that the EBV VCA antibody titer was significantly lower and the serum IFN-γ concentration higher in EBs. A higher titer suggests that cellular immune function was less effective in keeping this latent virus quiescent in the EFs. Other evidence comes from the *ex vivo* cultures in that IL-10 production was lower in post-partums compared to controls, suggesting suppression of Th2 immunity. Lymphocyte proliferation was higher in post-partums for PHA, suggesting lymphocyte activation. The remarkable differences in lymphocyte populations between post-partums and controls suggest several possible processes. A possibility is increased extravasation of these cytotoxic cells into tissues such as the involuting uterus. The higher levels of acute phase reactants and proinflammatory cytokines suggest the possibility that there may be up-regulated endothelial adhesion molecules in post-partum women. TNF-α is an endothelial activator[22] and it is higher in post-partums. Interestingly, the CD56 level was inversely correlated with TNF-α in the data ($r = -0.26$, $P = 0.002$), supporting this mechanism.

The innate immune response is responsible for reacting with and destroying pathogens and damaged cells through inflammatory processes and post-partum women appears to have an activated innate response. This may help screen out fetal chimeric cells, microorganisms, and damaged cells, and in turn protect her (and thus her infant) from infection. The higher IL-10, cortisol and ACTH levels in post-partum women suggest that there are measures to balance the negative and tissue damaging effects of excessive inflammation. In rats, it has been noted that lactation improves wound healing.[23] Wounded lactating dams healed faster and there was a differential expression of proinflammatory cytokines and chemokines in the wounded tissue compared to non-lactating controls. On the other hands it is well known that certain autoimmune and inflammatory diseases increase in the post-partum and this may be in part be due to the innate and specific immune activation that appears to be associated with the post-partum.[12] Breastfeeding appears to provide an additional boost to these processes, but the post-partum period, regardless of feeding method, seems to be oriented towards heightened and activated innate and specific immune defenses. While breastfeeding provides an additional level of potential protection for these mothers and their infants, formula feeding mother also appear to be different than control women in innate and specific immunological processes. Formula feeding mothers are obviously not comparable to the virgin controls generally used in animal studies of lactation. These mothers are engaged in caregiving and nurturant activities and are neuroendocrinologically probably more like their breastfeeding counterparts than control women.

Mothering and lactating may be protected states. In past centuries women lactated for much longer periods than do modern women, and there are probably many evolutionary benefits inherent in the physiology of the post-partum.

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