In the Puerperium, Primiparae Exhibit Higher Levels of Anxiety and Serum Peptidase Activity and Greater Immune Responses Than Multiparae

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Background: Delivery is accompanied by increases in anxiety levels that are significantly related to an activation of the inflammatory response system (IRS), as indicated by increases in the serum concentrations of interleukin-6 and the interleukin-1 receptor antagonist (IL-1RA) and increases in the activity of prolyl endopeptidase (PEP), a cytosolic endopeptidase that cleaves peptide bonds on the carboxyl side of proline in behaviorally active neuropeptides involved in anxiety. Primiparae may show an immune responsivity differing from that in multiparae. The aims of this study were to examine whether there are differences in anxiety levels, the IRS, and serum PEP values between primiparae and multiparae before and after delivery.

Method: We administered the Spielberger State-Trait Anxiety Inventory (STAI) to and assessed serum IL-1RA and soluble CD8 (sCD8) concentrations and serum PEP activity in 48 primiparae and 48 multiparae at the end of term and 1 and 3 days after delivery. Data were gathered in 1996 and 1997.

Results: We found that in primiparae ($p = .001$), but not in multiparae ($p = .6$), there was a significant increase in the STAI score 3 days after delivery and that primiparae had significantly higher STAI scores than multiparae 3 days after delivery ($p = .01$). Primiparae showed significantly higher serum IL-1RA levels than multiparae 1 ($p = .003$) and 3 ($p = .02$) days after delivery, but not before delivery. Primiparae also had lower serum sCD8 and higher serum PEP activity than multiparae before and after delivery.

Conclusions: The results suggest that primiparae suffer greater anxiety and have a different neuroimmune responsivity than multiparae and that the increased anxiety levels in primiparae may be related to changes in the IRS and serum PEP activity.

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A high number of parturients suffer from "maternity blues" and postpartum depression in the early puerperium and in the first months after delivery, respectively.[1] Maternity blues occurs in the first few days after delivery and is a mild, transient condition characterized by crying spells, irritability, anxiety, sleep disturbances, somatic symptoms, and emotional lability.[1,2] Three days after delivery, 23% to 25% of puerperae suffer from transitory depressive symptoms and another 23% to 25% are anxious.[3,4] Postpartum depression generally occurs later, i.e., within the first month after delivery and for a smaller percentage of women, within the next few months.[1]

It is generally accepted that in the early puerperium, there is an increased inflammatory responsivity in the serum.[5–9] This is indicated by, for example, increased serum concentrations of interleukin-6 (IL-6), suggesting an activation of the inflammatory response system (IRS).[4,9] The early puerperium is also characterized by increased serum levels of the soluble interleukin-1 receptor antagonist (IL-1RA), which is mainly derived from monocytes and is released in vivo during inflammation.[10] The above findings suggest an activation of the monocytic arm of cell-mediated immunity in the early puerperium. The rapid decline in other immunosuppresive peptides, such as the leukemia-inhibitory factor receptor, corticotropin-releasing hormone, and placenta-derived immunosuppressive proteins, in the early puerperium indicates that women in the early puerperium have a lowered anti-inflammatory capacity in the blood as compared with prepartum values.[11–13] We were the first to report that parturients who developed postpartum anxious symptoms had significantly higher serum IL-6 and IL-1RA concentrations than parturients who did not experience increased anxiety. Since monocytic cytokines such as IL-6 may induce depressive symptoms, depression, and anxiety, these findings suggest that activation of the IRS in the early puerperium may be causally related to increased anxiety levels.[9]

Anxiety in the early puerperium is also related to increased activity of serum prolyl endopeptidase (PEP, also known as EC 3.4.21.26 and post-proline cleaving enzyme) and prolyl oligopeptidase.[4] PEP is a cytosolic endopeptidase that cleaves peptide bonds on the carboxyl side of proline in proteins of relatively small molecular

mass.[14–16] PEP degrades and processes several behaviorally active neuropeptides, such as arginine vasopressin (AVP), thyrotropin-releasing hormone (TRH), substance P, oxytocin, bradykinin, and neurotensin.[17] It is thought that PEP may play a role in the pathophysiology of unipolar and bipolar disorder and in the working mechanism of mood stabilizers.[18,19]

There is some evidence that primiparous mothers have, 1 month postdelivery, different major psychological concerns than multiparous mothers.[20] Some months after delivery, primiparae may experience more stress and depression-related symptoms then multiparae.[21] Other data suggest that at the time of delivery primiparae have a suppressed lymphoproliferative response as compared with multiparae.[22]

The aims of the present study were to examine whether (1) primiparae show more anxiety in the early puerperium than multiparae and whether primiparae have a higher prevalence of postpartum depression than multiparae; and (2) primiparae show significantly higher serum PEP activity in the early puerperium than multiparae and show significant signs of IRS activation, such as increased serum IL-1RA, and lower serum concentrations of the suppressor/cytotoxic T-cell antigen soluble CD8 (sCD8). IL-1RA is released in vivo during inflammation and is produced by monocytes/macrophages.[23] IL-1RA is a pure IL-1 receptor antagonist[24] that inhibits the biological activities of IL-1.[10] sCD8 is secreted by activated T lymphocytes, such as activated CD8+ T cells, and is another marker of cell-mediated immunity.[25]

METHOD

Subjects

Ninety-six healthy pregnant women participated in this study. They were admitted to the hospital (Ziekenhuis Oost Limburg [ZOL], Genk, Belgium) for delivery. They were consecutively admitted with no selection based on previous miscarriages, parity, or gravity. We excluded pregnant women with preeclampsia, signs of infection before delivery, ruptured membranes for more than 12 hours, or a secondary cesarean section after labor and women who went into labor prematurely (< 37 weeks). Exclusionary criteria for patients were (1) any present Axis I psychiatric disorder (to exclude interference with the delivery-induced increases in psychopathology) and (2) a past psychiatric Axis I disorder, except mood disorders (including unipolar and bipolar depression) as assessed by means of DSM-IV criteria[26] using the Structured Clinical Interview for DSM-III-R (SCID).[27] We excluded some women who still suffered from anxiety disorders (such as obsessive-compulsive disorders and generalized anxiety disorder) in the weeks prior to delivery, and, consequently, no more subjects with a life history of anxiety disorders were included in the present study. We omitted subjects who used major psychotropic medications, including antidepressant and antipsychotic drugs, and those with medical disorders. All women were medically healthy and had normal physical examination results and normal results on blood tests such as aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, hematocrit, serum electrolytes, blood urea, and creatinine. Moreover, all subjects were free of drugs known to interfere with neuroimmune functions and free of acute infectious or inflammatory reactions for at least 2 weeks prior to the study. The study protocol was approved by the institutional review board of the ZOL, Genk, Belgium. All subjects gave written informed consent after the study design was fully explained. Data were gathered in 1996 and 1997.

Procedures

The 96 parturients were divided into 2 groups on the basis of parity, i.e., primiparae (N = 48) versus multiparae (N = 48). Serum samples for the assay of IL-1RA and sCD8 levels and PEP activity were collected at 8:00 a.m. (± 30 min) 3 to 6 days before delivery (last ambulatory visit 3 to 6 days prior to the anticipated date of delivery) and 1 and 3 days after delivery (collected in the hospital). At the same time, the women completed the Spielberger State-Trait Anxiety Inventory (STAI).[28] The 3 STAI scores in the baseline and 2 postpartum conditions were complete in 91 of the parturients, i.e., 43 of the primiparae and all of the multiparae. Women were divided into groups on the basis of changes in STAI scores from the prepartum to the third day postpartum, as defined by the quartile 75 values of the residualized STAI scores (computed by means of regression analyses with the postpartum scores 3 days after delivery as dependent variable and the prepartum scores as explanatory variable).

Six to 10 months after delivery, subjects were interviewed over the telephone by a psychiatrist who administered a structured interview based on the SCID[27] to assess the occurrence of postpartum depression within the first 3 months after delivery. Telephone interviews to assess a past history of major depression according to DSM criteria are commonly used in epidemiologic studies.[29] These interviews were complete in 71 women.

IL-1RA was quantified by means of an enzyme-linked immunosorbent assay (ELISA) method (Eurogenetics, Tessenderlo, Belgium), which is an enzyme-linked immunoassay (EIA) based on polyclonal antibodies, and standardized using recombinant human IL-1RA (AB-280-NA, R&D Systems, Minneapolis, Minn.). The sensitivity of the assay was 0.1 ng/mL. The intra-assay coefficients of variation (CVs) were 9.8%, 7.2%, and 6.1% at the 0.150 ng/mL, 0.350 ng/mL, and 1.0 ng/mL (N = 21) secretion ranges, respectively. The sCD8 assay was performed with the sCD8 EIA (Eurogenetics, Tessenderlo, Belgium), which is a monoclonal antibody-based sand-

wich EIA. The sensitivity of the assay was 35 U/mL. The intra-assay CV was lower than 8%. Serum PEP activity was determined using a fluorimetric method[30] with the synthetic substrate Z-glycyl-prolyl-4-methylcoumarinyl-7-amide (Bachem Feinchemikalien AG, Bubendorf, Switzerland). One unit (U) of PEP activity was defined as enzyme catalytic activity that releases 1 μmol 7-amino-4-methylcoumarin in 1 minute under the assay conditions. The intra-assay CV in our laboratory is 4.8%. To minimize effects of interassay analytical variability, all serum specimens were assayed in a single run with a single lot number of reagents and consumables employed by a single operator.

Statistical Analysis

Repeated-measures analyses of variance (ANOVAs) were employed to assess (1) the between-subject variability with effects of classification, i.e., primiparae versus multiparae; (2) the within-subject variability with the baseline and 2 postpartum conditions as time factor; and (3) 2-way interactions between time and group. The results of repeated-measures ANOVAs were corrected for sphericity. Tests on simple effects were carried out to examine significant time effects or significant 2-way interactions between time and group. Tests on simple effects were also used to examine the effects of time in primiparae and multiparae separately and the differences between the groups in the variables at the 3 timepoints.[31] Group mean differences were checked with ANOVAs. The independence of classification systems was ascertained by means of analysis of contingence tables (chi-square test). Relationships between variables were assessed by means of Pearson product moment correlation coefficients or through multiple regression analyses.

RESULTS

There were 22 women with a change in STAI score ≥ quartile 75 values and 69 women with a change in STAI score < quartile 75 values. The delta STAI values from baseline to 3 days after delivery were significantly higher ($F = 53.7$, $df = 1,89$; $p < .0001$) in those with a change ≥ quartile 75 values (mean = 6.9 ± 2.8) than in those with a change < quartile 75 values (mean = 0.5 ± 3.6).

Figures 1 through 4 show the STAI score, serum IL-1RA and sCD8 levels, and serum PEP activity in the primiparae versus multiparae before and after delivery. A repeated-measures ANOVA with primiparae versus multiparae as factor and STAI score as dependent variable showed a significant effect of time ($F = 2.6$, $df = 2,150$; $p = .005$). Analyses of simple effects showed that there were (1) significant effects of time on STAI values in primiparae ($F = 6.9$, $df = 2,162$; $p = .001$), but not in multiparae ($F = 0.9$, $df = 2,150$; $p = .6$), and (2) significantly higher STAI values in primiparae than in multiparae 3 days ($F = 6.6$, $df = 1,243$; $p = .01$) after delivery, but not before delivery or 1 day after delivery. A Dunn test showed that the STAI score was significantly higher in primiparae than in multiparae ($t = 2.54$, $p = .01$). The number of women with a change in STAI score ≥ quartile 75 values was significantly greater ($\chi^2 = 7.6$, $df = 1$, $p = .006$) in primiparae than in multiparae (37.2% vs. 12.5%).

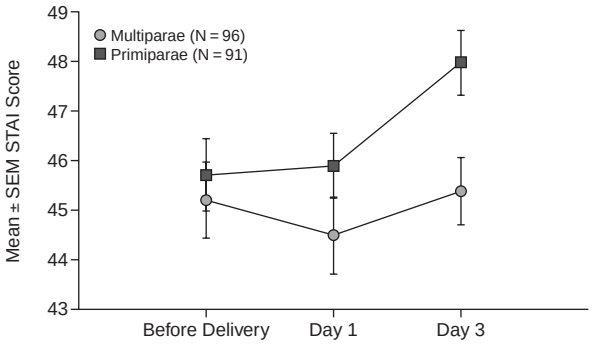
A repeated-measures ANOVA with primiparae versus multiparae as factor and serum IL-1RA level as dependent variable showed a significant effect of time ($F = 14.9$, $df = 2,118$; $p < .001$). Analyses of simple effects showed (1) significant effects of time on serum IL-1RA values in primiparae ($F = 14.5$, $df = 2,130$; $p < .001$), but not in multiparae ($F = 2.9$, $df = 2,130$; $p = .06$), and (2) significantly higher serum IL-1RA concentrations in primiparae than in multiparae 1 ($F = 9.4$, $df = 1,195$; $p = .003$) and 3 ($F = 5.7$, $df = 1,195$; $p = .02$) days after delivery, but not before delivery ($F = 0.5$, $df = 1,195$; $p = .5$). A Dunn test showed significantly higher serum IL-1RA concentrations in primiparae than in multiparae ($t = 2.80$, $p = .006$).

A repeated-measures ANOVA with primiparae versus multiparae as factor and serum sCD8 as dependent variable showed a significant effect of time ($F = 16.5$, $df = 2,127$; $p < .001$). Analyses of simple effects showed that there were (1) significant effects of time on serum sCD8 values in primiparae ($F = 6.3$, $df = 2,132$; $p = .002$) as well as in multiparae ($F = 10.6$, $df = 2,132$; $p = .0001$); and (2) significantly lower serum sCD8 in primiparae than in multiparae 1 ($F = 5.0$, $df = 1,198$; $p = .02$) and 3 ($F = 7.1$, $df = 1,198$; $p = .008$) days after delivery but not before delivery ($F = 3.5$, $df = 1,198$; $p = .06$). A Dunn test showed significantly lower serum sCD8 values in primiparae than in multiparae ($t = 3.91$, $p = .0003$).

A repeated-measures ANOVA with primiparae versus multiparae as factor and serum PEP activity as dependent variable showed a significant effect of time ($F = 8.8$, $df = 2,109$; $p = .0002$). Analyses of simple effects showed that there were (1) significant effects of time on serum PEP activity in primiparae ($F = 6.3$, $df = 2,134$; $p = .002$) and multiparae ($F = 5.3$, $df = 2,134$; $p = .006$) and (2) significantly higher serum PEP activity in primiparae than in multiparae before delivery ($F = 4.7$, $df = 1,201$; $p = .02$) and 1 ($F = 5.9$, $df = 1,201$; $p = .02$) and 3 ($F = 4.7$, $df = 1,201$; $p = .02$) days after delivery ($F = 3.5$, $df = 1,198$; $p = .06$). A Dunn test showed significantly higher serum PEP values in primiparae than in multiparae ($t = 3.92$, $p = .0003$).

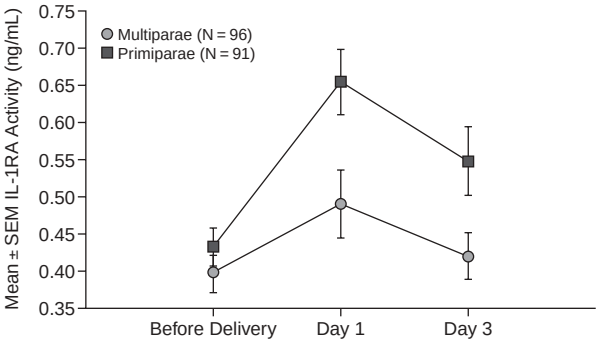
There were significant differences ($F = 11.4$, $df = 1,94$; $p = .001$) in age between primiparae (mean = 26.6 ± 3.6 years) and multiparae (mean = 28.9 ± 3.1 years). There were, however, no significant correlations between age and STAI scores, serum IL-1RA or sCD8 levels, or PEP activity at any of the 3 timepoints, which shows that there was no significant effect of age on the results. The differ-

Figure 1. STAI Scores in Primiparae and Multiparae 3 to 6 Days Before Delivery and 1 and 3 Days After Delivery



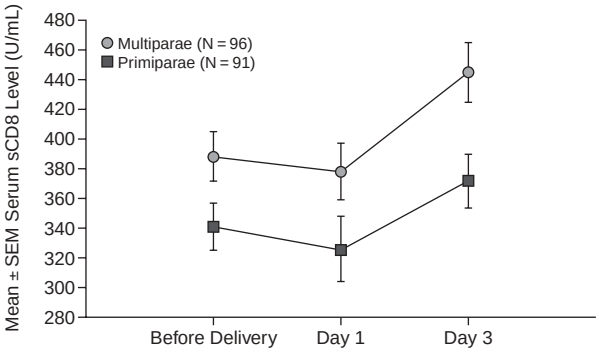
Abbreviation: STAI = State-Trait Anxiety Inventory.

Figure 2. Serum IL-1RA Levels in Primiparae and Multiparae 3 to 6 Days Before Delivery and 1 and 3 Days After Delivery



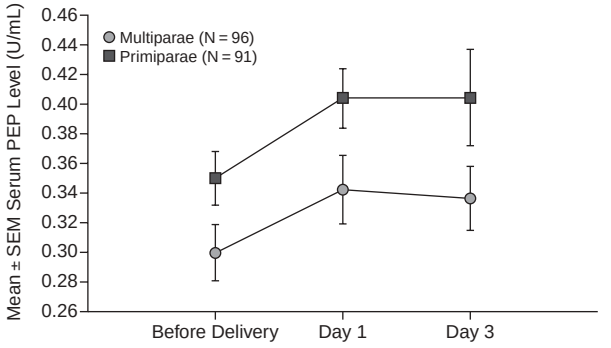
Abbreviation: IL-1RA = interleukin-1 receptor antagonist.

Figure 3. Serum sCD8 Levels in Primiparae and Multiparae 3 to 6 Days Before Delivery and 1 and 3 Days After Delivery



Abbreviation: sCD8 = soluble CD8.

Figure 4. Serum PEP Levels in Primiparae and Multiparae 3 to 6 Days Before Delivery and 1 and 3 Days After Delivery



Abbreviation: PEP = prolyl endopeptidase.

ences on the STAI and in serum IL-1RA and sCD8 levels and PEP activity between primiparae and multiparae were not significantly associated with maternal and labor variables such as duration of pregnancy and labor, labor induction with intravenous oxytocin or intravaginal prostaglandin (yes or no), amniotomy (spontaneous vs. induced), breastfeeding (yes or no), or postpartum complications (such as malleolar edema and use of antibiotics for possible infection). Although the number of women with a change in STAI score ≥ quartile 75 values was significantly greater ($\chi^2 = 5.9$, df = 1, p = .01) in cases of induced labor versus cases without (42.3% vs. 17.7%), there were no significant associations between induced labor and the differences in the STAI and in serum IL-1RA and sCD8 levels and PEP activity between primiparae and multiparae.

DISCUSSION

The first major finding of this study is that primiparae experience significantly greater anxiety levels 3 days after delivery than multiparae. It is noteworthy that differences in STAI score between primiparae and multiparae existed at 3 days postpartum but not during the immediate postpartum period (day 1) or antenatally. Therefore, primiparae not only suffer from more anxiety 3 days after delivery than multiparae, but also show a significantly greater increase in anxiety levels from the prepartum to the postpartum period. However, the increased anxiety that primiparae experience may have caused a selection bias affecting the level of anxiety in multiparous women, since the primiparae who experience the most anxiety and/or depression may choose not to have more children. In addition, in the present study, we only scored the changes in the STAI as a measure of postpartum anxiety, whereas it would also have been helpful to know which patients met the criteria for postpartum anxiety disorders.

Nevertheless, our findings that primiparae had significantly higher STAI scores after delivery than multiparae extend previous findings. Three days after delivery, 23% to 25% of the puerperae suffer from transitory depressive symptoms and another 23% to 25% are anxious.[3,4] It has

been shown that primiparae have, 1 month postdelivery, different major psychological concerns than multiparous mothers.[20] The most frequently identified concerns of primiparae are baby feeding, fatigue, baby behaviors, return of the figure, breast soreness, limitation of visitors, regulation of demands, and growth and development of the baby. Sato et al.[21] found that primiparae experience more rearing-related stress than multiparae. Kendell et al.,[32] on the other hand, could find no significant differences in ratings of depression, tears, or lability between primiparae and multiparae.

In addition, there are delivery-related differences between primiparae and multiparae that should be considered in the interpretation of the results. (1) The incidence of preeclampsia is significantly higher in primiparae (14.1%) than in multiparae (5.7%).[33] (2) Multiparae have a larger incidence of spontaneous deliveries, whereas directed and induced deliveries are more frequent in primiparae.[34] (3) The mean duration of labor is significantly longer in primiparae (14.9 h) than in multiparae (10.0 h).[35] (4) Primiparae have a higher risk of low birth weight in their infants than multiparae.[36] (5) Moreover, multiparae are significantly older then primiparae. In the present study, however, we controlled for age and delivery-related variables such as duration of pregnancy and labor, induced labor and amniotomy, breastfeeding, and postpartum complications, such as malleolar edema and use of antibiotics for possible infection. In addition, no women with preeclampsia were included in the present study.

The second major finding of this study is that primiparae have significantly higher serum IL-1RA concentrations 1 and 3 days after delivery and that primiparae have lower serum sCD8 and higher serum PEP activity before and after delivery than multiparae. These findings suggest that primiparae have an exaggerated monocytic responsivity (higher serum IL-1RA concentrations) in the early puerperium and are characterized by lowered T-cell activity (serum sCD8) and increased serum PEP activity.

A few data in the literature suggest that primiparae may have a distinct immune responsivity from that of multiparae. Pregnant women and in particular primiparae are therefore predisposed to develop malaria infection.[22] The proliferation of peripheral blood and placental lymphocytes to malaria-specific antigens, *Candida*, and purified protein derivatives is selectively suppressed in primiparae as compared with multiparae.[22] Moreover, malaria reduces fetal growth in primiparae more than in multiparae.[37] It was hypothesized that the greater susceptibility to malaria infection in primiparae may be caused by the presence of immunosuppressive factors in their blood.[22] Our results show that in primiparae there may be an activation of the IRS with simultaneous signs of immune activation and immunosuppression. Thus, while increased serum IL-1RA levels indicate a greater monocytic activation, IL-1RA displays immunosuppressive activities.[10] Moreover, the decreased serum levels of the sCD8 suppressor/cytotoxic T-cell antigen may indicate a defect in the early stages of CD8+ T-lymphocyte activation.[38] Future research should focus on differences in other markers of immunoresponsivity between primiparae and multiparae, such as the production of proinflammatory and anti-inflammatory cytokines by stimulated whole blood and acute-phase proteins.

As reported previously,[9] parturients who developed postpartum anxious symptoms had significantly higher serum IL-1RA concentrations than parturients without an increase in anxiety, suggesting that activation of macrophages/monocytes in the postpartum period is related to anxiety.[9] The increased anxiety levels in students with examination stress are accompanied by increased serum IL-1RA concentrations.[39] Since monocytic cytokines such as IL-1, IL-6, and tumor necrosis factor-α may induce depression and anxiety,[40] it may be hypothesized that the increased monocytic activity in the postpartum period in primiparae may have caused the higher anxiety levels.

Previously, we reported that serum PEP activity is significantly higher 1 and 3 days after delivery than before delivery and that parturients who suffer from increased anxiety levels in the postpartum period have significantly increased serum PEP activity compared with puerperae without anxiety.[4] One explanation is that PEP plays a key role in the final degradation and processing of behaviorally active hormones and neuropeptides, such as TRH, substance P, neurotensin, oxytocin, and AVP,[17] which play a pivotal role in anxiety, emotion, stress responsivity, avoidance behavior, and social interactions.[4,18] Oxytocin has anxiolytic properties and stimulates social behavior and interactions; neurotensin is involved in the activity of dopaminergic neurons, which mediate behaviors motivated by positive reinforcers; substance P has positive reinforcing capacities; and AVP facilitates positive conditioning, improves social recognition, and increases social interactions.[4,18] Therefore, it may be hypothesized that the higher anxiety levels in primiparae are related not only to a higher monocytic activity but also to higher serum PEP activity in primiparae than in multiparae.

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