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*J Immunol* 2005; 174:3765-3772; doi: 10.4049/jimmunol.174.6.3765

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Prolonged Lymphopenia, Lymphoid Depletion, and Hypoprolactinemia in Children with Nosocomial Sepsis and Multiple Organ Failure

Kate A. Felmet,* Mark W. Hall,‡ Robert S. B. Clark,* Ronald Jaffe,† and Joseph A. Carcillo2*

Lymphopenia and lymphoid depletion occur in adults dying of sepsis. Prolactin increases Bcl-2 expression, suppresses stress-induced lymphocyte apoptosis, and improves survival from experimental sepsis. We hypothesized that prolonged lymphopenia, lymphoid depletion, and hypoprolactinemia occur in children dying with sepsis and multiple organ failure (MOF). Fifty-eight critically ill children with and 55 without MOF admitted to a university hospital pediatric intensive care unit were enrolled in a prospective, longitudinal, observational clinical study. Prolactin levels and absolute lymphocyte count were measured on days 1, 3, 7, 14, and 21. Lymph node, thymus, and spleen autopsy specimens were examined for lymphoid depletion, with immunohistochemical staining for CD4, CD20, and CD21 and for lymphoid apoptosis. Prolonged lymphopenia (absolute lymphocyte count < 1000 for >7 days) occurred only in children with MOF (29 vs 0%, p < 0.05) and was associated independently with nosocomial infection (odds ratio (OR), 5.5, 95% confidence interval (CI), 1.7–17, p < 0.05), death (OR, 6.8, 95% CI, 1.3–34, p < 0.05), and splenic and lymph node hypocellularity (OR, 42, 95% CI, 3.7–473, p < 0.05). Lymphocyte apoptosis and ante/postmortem infection were observed only in children with lymphoid depletion. Prolonged hypoprolactinemia (>7 days) was more common in children with MOF (17 vs 2%, p < 0.05) and was associated independently with prolonged lymphopenia (OR, 8.3, 95% CI, 2.1–33, p < 0.05) and lymphoid depletion (OR, 12.2, 95% CI, 2.2–65, p < 0.05). Prolonged lymphopenia and apoptosis-associated depletion of lymphoid organs play a role in nosocomial sepsis-related death in critically ill children. Prolonged hypoprolactinemia is a previously unrecognized risk factor for this syndrome. The Journal of Immunology, 2005, 174: 3765–3772.

Stress-induced lymphocyte apoptosis is thought to be mediated, in part, through the adrenocorticotropic hormone-cortisol axis. Prolactin is the counterregulatory stress hormone produced by the anterior pituitary and by lymphocytes and monocytes, which prevents cortisol/stress-induced lymphocyte apoptosis through increased Bcl-2 production (9–11). Anterior pituitary dysfunction has been documented in adult critical illness, with respect to the growth hormone, the thyroid-stimulating hormone, and the gondadotropin-releasing hormone, but the prolactin axis has not been investigated in detail (12). Chaudry and colleagues (13, 14) found that experimentally induced hemorrhagic shock caused decreased lymphocyte proliferation, increasing susceptibility to death from cecal ligation, and puncture-induced sepsis. Administration of either prolactin or a prolactin secretagogue (metoclopramide) reversed this process, preventing hemorrhage-induced suppression of splenocyte proliferation and cytokine release capacity and improving survival from subsequent cecal ligation and puncture-induced sepsis (13, 14). Although this phenomenon has not been examined in humans, it appears plausible biologically that a state of prolonged hypoprolactinemia could predispose critically ill patients to stress-induced lymphopenia and lymphoid depletion.

In the present study, we hypothesized that critically ill children with prolonged lymphopenia are more likely to develop nosocomial infection or apoptosis-associated lymphoid depletion or to die. We also hypothesized that prolonged hypoprolactinemia predisposes critically ill children to the development of prolonged lymphopenia and apoptosis-associated lymphoid depletion.

Materials and Methods

The local institutional review board approved the study. Informed consent was obtained from the parents of children participating in the study.
graded by the blinded pathologist and defined as severe when... lymphocyte apoptosis. Lymphocyte depletion in each tissue was... and lymphocyte apoptosis. Lymphocyte depletion in each tissue was... in each tissue was... thymus, and spleen were examined for evidence of lymphocyte depletion... in... who died and underwent autopsy, samples of lymph node, thymus, and spleen were examined for evidence of lymphocyte depletion... In study patients who required an arterial line within 24 h of pediatric intensive care unit (PICU) admission. Blood samples were collected on days 1, 3, 7, 14, and 21. The sample collection was terminated at the time the invasive access was discontinued, the patient died, or the patient was discharged from the intensive care unit... Two patient cohorts were collected. The first cohort, collected between March 1999 and June 2000, enrolled consecutive critically ill children who required an arterial line within 24 h of admission. The second cohort, collected between March 2001 and June 2001, enrolled consecutive critically ill children who required an arterial line within 24 h of pediatric intensive care unit (PICU) admission. Blood samples were collected on days 1, 3, 7, 14, and 21. The sample collection was terminated at the time the invasive access was discontinued, the patient died, or the patient was discharged from the intensive care unit.

Data collection
In all patients, a morning blood sample was drawn in a heparinized tube and immediately spun. The serum was decanted and frozen at −20°C. At the end of the study, prolactin levels were measured in all samples by an automated chemiluminescent assay (Immulite; Diagnostic Products Corporation) Hypoprolactinemia was defined as <2.5 ng/ml in children >6 mo of age and <20 ng/ml in children <6 mo of age (16, 17). Prolonged hypoprolactinemia was defined as low prolactin levels for >1 wk or until death.

On the day of admission, a pediatric risk of mortality (PRISM) score was calculated for all patients. At the time of collection, OFI, pressor type and dose, use of metoclopramide, use of steroids, and other immune suppressive agents were recorded. Absolute lymphocyte counts (ALC) were recorded as measured by a routine hospital laboratory complete blood count. Lymphopenia was defined as ALC <1000 (18–21). Lymphopenia was defined as prolonged when lasting >1 wk or until death. Nosocomial infection was defined by a positive culture from blood, urine, or endotracheal tube with increased white blood cells (>12,000) occurring between days 5 and 25 (mean, 11 days of PICU admission) and requiring antibiotics per the attending physician.

Screening for lymphocyte depletion and apoptosis
In study patients who died and underwent autopsy, samples of lymph node, thymus, and spleen were examined for evidence of lymphocyte depletion and lymphocyte apoptosis. Lymphocyte depletion in each tissue was graded by the blinded pathologist and defined as severe when <75% of... to determine which factors were... normal lymphocyte population in each tissue was seen. Paraffin-embedded sections of lymphocyte, thymus, and spleen were evaluated for the presence of lymphocyte apoptosis by TUNEL staining with a concomitant nuclear stain. Cell populations were identified using immunohistochemical staining for CD4 (T lymphocytes), CD20 (B lymphocytes), and CD21 (dendritic cells).

TUNEL staining was performed as follows. Slides were deparaffinized and rehydrated, then treated with proteinase for 30 min (Boehringer Mannheim) to increase membrane permeability. Enzyme activity was quenched with a solution of 3% hydrogen peroxide in 30% methanol for 30 min. Sections were incubated with a mixture of recombinant TdT (Invitrogen Life Technologies) and biotin-16-deoxy-uridine-5-triphosphate (Roche) for 2 h. Sections were then incubated for 1 h with a streptavidin-conjugated fluorescent dye (Alexa Fluor 488; Molecular Probes) and the nuclear dye bis-benzamide for 1 min (Sigma-Aldrich). TUNEL-positive cells were identified by the presence of fluorescence in the spectrum of bis-benzimid and streptavidin colocalized to the same cell. Tissues were defined as positive for apoptosis when more than three TUNEL-positive cells were seen per high-powered field.

Immunohistochemical staining for cluster of differentiation markers was performed as follows. Slides were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide for 30 min. Slides were microwaved for 10 min in the buffer recommended by the manufacturer for each primary Ab. After the application of a blocking agent, slides were incubated with mAbs against human CD4, CD20, and CD21 for 1 h at room temperature (Vector Laboratories). Slides were incubated with a biotinylated secondary Ab for 30 min, then developed using an avidin-biotinylated enzyme complex with a vasoactive intestinal peptide substrate (Vector Laboratories).

Statistical analysis
Data were analyzed using SigmaStat and Stata. Data involving two groups over time were analyzed using two-factor ANOVA for ranks. Data involving two groups over time were analyzed using two-factor ANOVA for ranks. Significance was accepted at \( p < 0.05 \). To determine which factors were...
associated with lymphopenia, lymphoid depletion, nosocomial infection, and death, we performed a univariate analysis with the following variables: age, presence of steroids, presence of immune suppression, prolonged hy- poprolactinemia, PRISM score, and dopamine exposure. Odds ratios (OR) were determined using all factors with \( p < 0.1 \) in a logistic regression analysis.

**Results**

**Patient demographics**

A total of 113 patients was enrolled. Primary admission diagnoses included sepsis (\( n = 35 \)), respiratory failure (\( n = 8 \)), postoperative (\( n = 14 \)), trauma (\( n = 7 \)), status postorgan transplantation (\( n = 28 \)), fulminant hepatic failure (\( n = 7 \)), and other (\( n = 17 \)). There were 62 males and 51 females with an average age of 7.5 years (range, 2 wk to 23 years) and a mean PRISM score of 10 (range, 0–26). There were 58 patients with MOF (135 patient days) and 55 patients without MOF (120 patient days). Among patients with MOF, the mean OFI at entry into the study was 2.9 compared with 0.05; Fig. 1). Lymphocyte counts were lower in patients with MOF than those without (median, 864 \( / \text{H9273} \) vs 1787, \( / \text{H11005} \), \( p < 0.001, \text{ Mann-Whitney rank-sum test} \). MOF patients remained lymphopenic over time, while the counts of patients without MOF increased (two-factor ANOVA for ranks; time \( \times \) group, \( p < 0.05; \) Fig. 1).

**Prolonged lymphopenia**

Forty-nine percent of the patients had lymphopenia for \( \geq 1 \) days and 15% had prolonged lymphopenia. Twenty-nine percent (17 of 58) of patients with MOF had prolonged lymphopenia vs 0 of 55 patients without MOF (\( p < 0.001, \chi^2 \) test). Lymphocyte counts were lower in patients with MOF than those without (median, 864 \( / \text{H9273} \) vs 1787, \( p < 0.001, \text{ Mann-Whitney rank-sum test} \). MOF patients remained lymphopenic over time, while the counts of patients without MOF increased (two-factor ANOVA for ranks; time \( \times \) group, \( p < 0.05; \) Fig. 1).

**Transient lymphopenia**

Transient lymphopenia for 3–7 days was associated with an increased likelihood of nosocomial infection (OR, 4.4, 95% confidence interval (CI), 1.2–15.4, \( p < 0.02 \)) independent of immune suppression or steroid use but not an increased likelihood of MOF or death. Prolonged lymphopenia was associated with secondary infection (OR, 5.5, 95% CI, 1.7–17, \( p < 0.05 \), MOF (\( p < 0.001, \text{ Fisher’s exact test} \), and death (OR, 6.8, 95% CI, 1.3–34, \( p < 0.05 \)) independent of steroid use, immune suppression, and risk of mor-

tality (PRISM score). Prolonged peripheral lymphopenia was an independent predictor of lymphocyte depletion at autopsy with an OR of 42.2 (95% CI, 3.7–473, \( p = 0.001 \). Patients with prolonged lymphopenia had normal neutrophil counts (Fig. 2).

**Lymphoid depletion and autopsy findings**

Sixteen patients died and 11 underwent autopsy. Median time from death to autopsy was 13 h (range, 3–39 h). A summary of autopsy findings can be found in Table I. Severe lymphocyte depletion (Fig. 3) was seen in eight of nine (89%) patients dying with MOF. The one patient who died with MOF without lymphocyte depletion had a rapid course with fulminant hepatic failure. Neither of the two patients who died without MOF showed evidence of lymphocyte depletion (\( p = 0.05, \text{ Fisher’s exact test} \). Lymph nodes and spleens of patients with MOF were hypocellular with sparse and atrophic lymphoid nodules. The thymuses in patients with MOF were hypocellular. The same tissues in the two patients who died without MOF showed normal cell populations. In the lymphocyte-depleted patients, seven of eight or 88% had autopsy evidence of unresolved nosocomial infection. Four of these patients grew multiple organisms from autopsy specimens. None of the three patients without lymphocyte depletion had infection before death or in autopsy cultures (\( p = 0.02, \text{ Fisher’s exact test} \).

**Table I. Autopsy findings**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cause of Death</th>
<th>Known Nosocomial Infection</th>
<th>Unrecognized Infection at Autopsy</th>
<th>Days from Onset of MOF to Death</th>
<th>Lymphocyte Depletion</th>
<th>Prolonged Low Prolactin?</th>
<th>Immune Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with MOF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Pulmonary hemorrhage</td>
<td>Micrococcus, Candida albicans, and CNS (blood)</td>
<td>Disseminated C. albicans</td>
<td>16 days</td>
<td>Yes</td>
<td>No</td>
<td>Cyclosporin</td>
</tr>
<tr>
<td>29</td>
<td>Refractory hypotension after heart transplant</td>
<td>None</td>
<td>None</td>
<td>13 days</td>
<td>Yes</td>
<td>Yes</td>
<td>Atgam</td>
</tr>
<tr>
<td>35</td>
<td>Acute intracranial hemorrhage</td>
<td>Enterobacter cloacae (blood)</td>
<td>Pseudomonas, Citrobacter, and Serratia (lung tissue)</td>
<td>16 days</td>
<td>Yes</td>
<td>No</td>
<td>Tacrolimus, high-dose steroids</td>
</tr>
<tr>
<td>38</td>
<td>Support limited-refractory hypotension and parainfluenza</td>
<td>Enterococcus (blood) and parainfluenza</td>
<td>Enterococcus fecalis and CNS (blood)</td>
<td>49 days</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>43</td>
<td>Withdrawal of support</td>
<td>C. albicans (lungs)</td>
<td>E. fecalis (blood)</td>
<td>16 days</td>
<td>Yes</td>
<td>Yes</td>
<td>Cyclosporin</td>
</tr>
<tr>
<td>44</td>
<td>Brain herniation</td>
<td>C. albicans (urine)</td>
<td>Disseminated C. albicans</td>
<td>9 days</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>46</td>
<td>Fulminant hepatic failure</td>
<td>None</td>
<td>None</td>
<td>2 days</td>
<td>No</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>53</td>
<td>Refractory hypotension</td>
<td>None</td>
<td>E. fecalis, Enterobacter aerogenes, and Staphylococcus epidermidis (blood), pseudomembranous colitis</td>
<td>34 days</td>
<td>Yes</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>57</td>
<td>Intracranial hemorrhage</td>
<td>None</td>
<td>Severe pseudomembranous colitis and C. albicans (mediastinum)</td>
<td>6 days</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>Patients without MOF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Pulmonary hypertensive crisis</td>
<td>None</td>
<td>None</td>
<td>2 days</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>32</td>
<td>Withdrawal of support</td>
<td>None</td>
<td>None</td>
<td>3 days</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Immunohistochemical staining revealed a reduction in numbers of lymphocyte cell lines in patients with lymphocyte depletion (Figs. 4 and 5). A decrease in the number and size of CD20-stained lymphoid follicles (zones of active B cell replication) in the spleens and lymph nodes of patients with lymphoid depletion was found in most patients with MOF. Individual MOF patients with lymphoid depletion also had a decrease in CD4-positive T cell staining. Patients dying without MOF had normal populations of all three cell lines.

TUNEL-positive apoptotic cells were seen in patients with MOF but not in those without MOF (Fig. 6). Fifty percent of thymuses, 57% of lymph nodes, and 89% of spleens were TUNEL positive in patients dying with MOF (p = 0.05, Fisher’s exact test). The median delay between death and tissue fixation was similar for patients with TUNEL-positive tissues compared with those without.

**Prolonged hypoprolactinemia, prolonged lymphopenia, and lymphoid depletion**

Fifty percent of PICU patients had one or more episodes of hypoprolactinemia and 11% had prolonged hypoprolactinemia. Prolonged hypoprolactinemia occurred more commonly in patients with MOF. Seventeen percent (10 of 58) of patients with MOF had prolonged hypoprolactinemia compared with 2% (1 of 55) of patients without MOF (p < 0.01, χ² test). Patients with prolonged hypoprolactinemia had lower peripheral lymphocyte counts (two-factor ANOVA for ranks, group, p < 0.05; Fig. 7).

In univariate analysis, age, steroid use, dopamine use, immune suppression, and prolonged hypoprolactinemia were associated with prolonged lymphopenia (p < 0.1). In multivariate analysis, prolonged hypoprolactinemia was associated independently with prolonged peripheral lymphopenia (OR, 8.3, 95% CI, 2.1–33, p = 0.003). Dopamine use was a risk factor for prolonged hypoprolactinemia with an OR of 7.3 (95% CI, 1.6–32.3), but it was not an independent risk factor for prolonged lymphopenia. Three patients received metoclopramide, each for 1 day only, and all three had normal prolactin levels and ALC.

In univariate analysis, age, steroid use, immune suppression, and prolonged hypoprolactinemia were associated with lymphoid depletion (p < 0.1). In multivariate analysis, prolonged hypoprolactinemia was associated independently with lymphoid depletion (OR, 12.2, 95% CI, 2.2–65, p = 0.01). Five of the eight patients with lymphoid depletion had prolonged hypoprolactinemia (Table I). Four of the eight patients with lymphoid depletion were treated with drugs that are known to contribute to lymphocyte loss: one patient received anti-thymocyte globulin (a lympholytic agent), one patient received tacrolimus, and two patients received cyclosporine A (a prolactin receptor antagonist). Only one of the eight patients with lymphoid depletion neither received a drug known to cause lymphopenia, nor experienced prolonged hypoprolactinemia.

**Discussion**

Three novel findings are presented in this study. First, a simple clinical laboratory test, an ALC < 1000, not only identified critically ill children at risk for nosocomial infection, but an ALC < 1000 for >7 days identified those at risk for death from uneradicated nosocomial infection. Second, apoptosis-associated B cell, T cell, and dendritic cell depletion was found in the lymphoid organs from children who died from MOF and uneradicated infection. Similar to adults, children dying from sepsis-induced...
MOF may do so, in part, because of lymphoid depletion. Apparently, lymphocyte depletion can be so profound that it impairs the child’s ability to eradicate infection, despite the normal neutrophil counts seen in these patients. Third, prolonged hypoprolactinemia may be a previously unrecognized risk factor contributing to the pathophysiology of lymphoid depletion syndrome. Prolonged lymphopenia, lymphoid depletion, and death from nosocomial sepsis may occur, in part, due to absence of the counterregulatory hormone that ameliorates stress-mediated immune cell apoptosis.

Absolute neutropenia has long been recognized as a risk factor for nosocomial sepsis in children and adults. Patients with absolute neutrophil counts < 500 and fever are treated empirically with broad spectrum antibiotics. If fever and neutropenia persists for >5 days, then antifungal therapy is added to the empiric regimen. Patients with prolonged neutropenia are also treated with growth factors or white blood cell transfusions to prevent or cure nosocomial sepsis. Persistent neutropenia, unresponsive to these therapies, is associated with death from nosocomial sepsis. Although absolute lymphopenia has long been taken for granted as an innocent bystander in patients with critical illness, the preponderance of clinical data suggests otherwise (4, 5, 20–22). The HIV and cancer chemotherapy literature has identified CD4 counts <1500 in infants, 1000 in 1- to 5-year-olds, 500 in 6- to 12-year-olds, and 200 in older children as a risk factor for nosocomial infection with viruses, fungus, and protozoa (23). Prophylaxis and empiric antimicrobial strategies are used as the standard of care in these populations. The primary immune deficiency and bone marrow transplantation literature recognize the importance of decreased CD20 counts to the development of hypogammaglobulinemia and an increased risk of nosocomial sepsis (24). These children are treated with i.v. Ig (IVIG) prophylaxis on a 3- to 4-wk basis to maintain IVIG levels >500 at all times. In our patient population, children with lymphopenia commonly had depressed CD4 and/or CD20 cell counts. Similar to clinical experience with neutropenia, transient lymphopenia is a risk factor for nosocomial infection. Prolonged lymphopenia is a risk factor for uneradicable nosocomial sepsis, MOF, and death.

Gurevitch et al. (25) previously reported lymphoid depletion at an autopsy in low birth weight infants who died with systemic Ag-related disease or sepsis. Hotchkiss et al. (7) similarly found lymphoid depletion in adults dying from sepsis. These patients had reduced lymphoid CD4 and CD20 cell numbers with the degree of reduction in cell numbers related to the duration of sepsis before death. Patients with sepsis <7 days had less depletion than patients with sepsis >7 days. These investigators also found >3% apoptotic cells in specimens with lymphoid depletion (7). In our study, all autopsy specimens were obtained from children who had sepsis-associated MOF for >7 days. The patterns of lymphoid depletion were similar to those reported by Gurevitch et al. (25) and Hotchkiss et al (7). There was a reduction in CD4, CD20, and CD21 cells with >3% of cells demonstrating apoptosis. It is possible that this profound depletion of immune cells needed for cell-mediated immunity, humoral immunity, and Ag presentation and MOF and two patients without. Patients without lymphoid depletion had more numerous and robust lymphoid follicles than those without. B, Immunohistochemical staining for T cells (CD4) in the periarteriolar zone of the spleens of two patients with lymphoid depletion and MOF and two patients without, showing a decrease in this cell population in patients with lymphoid depletion. Magnification is ×200. C, Immunohistochemical staining for mature dendritic cells (CD21) in the spleen of one patient with lymphoid depletion and MOF (patient 44) and one patient without (patient 12), showing decreased numbers in the lymphoid-depleted patient.

FIGURE 5. Immunohistochemical staining for B cells, T cells, and follicular dendritic cells. A, Immunohistochemical staining for B cells (CD20) in splenic lymphoid follicles of two patients with lymphoid depletion and
processing contributed to uneradicable infection in these patients. Experimental evidence supporting the role of lymphocytes in bacterial killing has been provided by Hotchkiss et al. (8), who demonstrated that administration of a polycaspase inhibitor to a rodent model of experimental sepsis increased lymphocyte counts, reduced bacterial counts, and improved survival.

The causes of prolonged lymphopenia and lymphoid depletion are likely multifactorial. Deficiencies in factors necessary for lymphocyte growth and proliferation, particularly zinc, may contribute to lymphopenia in patients with prolonged marginal nutrition (26). Iatrogenic immune suppression also plays a role as demonstrated by the contribution of steroids and immune suppressants to the development of lymphopenia in our patients. However, our study suggests that previously unappreciated prolonged hypoprolactinemia may be another important contributor to lymphopenia and lymphocyte apoptosis. As both a growth factor and an antiapoptotic hormone, prolactin may support circulating lymphocyte numbers in humans as it does in animal models of stress. Withdrawal of the proliferation cofactor IL-2 has been shown to induce apoptosis-by-neglect (27). The absence of prolactin, which is a necessary cofactor for IL-2-mediated lymphocyte proliferation, may have a similar effect (28). Prolactin is known to protect against glucocorticoid-induced lymphocyte apoptosis in vitro and in animal models (29, 30), and levels of glucocorticoids commonly seen in stressed patients have been shown to cause lymphocyte apoptosis (31, 32). Because prolactin release occurs simultaneously with stress-induced hypothalamic/pituitary/adrenal axis activation, it may protect the organism from the immune-suppressive effects of stress cortisol. Prolactin can mediate these effects, in part, through regulation of expression of antiapoptotic genes (particularly BcL-2) by lymphocytes in vitro (33, 34). Overexpression of BcL-2 in transgenic mice has been shown to decrease lymphocyte apoptosis and improve survival from sepsis (35).

Patients undergoing stress from uncomplicated surgery or moderate infections have increased prolactin levels (36, 37); however, our critically ill population did not show this stress response. Pituitary prolactin release is inhibited tonically by endogenous dopamine release by the hypothalamus. When dopamine infusions are used in the intensive care unit, prolactin release is inhibited strongly by dopamine at dosage levels as low as 0.1 μg/kg/min (38). We found that dopamine infusion use was associated with hypoprolactinemia in our patients; however, hypoprolactinemia was also observed in patients who did not receive dopamine infusions. Loss of normal patterns of secretion of other pituitary hormones have been demonstrated in patients with prolonged critical

FIGURE 6. TUNEL staining for apoptosis. A representative field of splenocytes stained with a blue nuclear stain and a green TUNEL stain in a patient with lymphoid depletion and MOF (patient 53) and one without (patient 12). TUNEL-positive apoptotic cells (arrows) are those in which the green and blue colocalize to the same cell. All images are at ×600 magnification.

FIGURE 7. Lymphocyte counts (ALC) in patients with and without prolonged hypoprolactinemia. Patients with prolonged hypoprolactinemia had lower lymphocyte counts ($p < 0.05$).
illness (12). Whether hypoprolactinemia in some critically ill patients is caused by increased endogenous hypothalamic dopamine production or other types of pituitary dysfunction remains to be studied.

Chaudry and colleagues (13, 14, 39) have addressed the relationship of prolactin, reduced lymphocyte proliferation, and susceptibility to death from sepsis in the experimental setting. These investigators induced hemorrhagic shock and caused reduced lymphocyte proliferation in mice. When subsequently challenged with sepsis using the cecal-ligation puncture model, survival was impaired; however, treatment with prolactin at the time of hemorrhage restored lymphocyte and macrophage function and improved survival from sepsis (14). This protection was also achieved with treatment with the prolactin secretagogue, metoclopramide, which acts as a partial dopamine antagonist. Metoclopramide increased prolactin levels, increased splenocyte proliferation, reduced systemic IL-6 production, and improved survival (13, 39). These findings provide biologic plausibility for the potential of prolactin and prolactin secretagogues to help reverse susceptibility to lymphoid depletion and nosocomial infection in critically ill patients. However, it is important to note that in vitro and animal studies suggest a biphasic effect of prolactin on the immune system similar to that seen in other target organs (40, 41). Hypoprolactinemia (in humans, <2.5 ng/ml; Ref. 17) or extreme hyperprolactinemia (in humans, approximately >200 ng/ml) are associated with immune-suppressive effects. Oberbeck et al. (42) recently demonstrated the immune-suppressive effects of high-dose prolactin in this study, exogenous prolactin administered at levels 60-fold higher than those seen during stress-induced release of the endogenous hormone lead to increased sepsis-induced mortality and impaired lymphocyte function (42).

In contrast, prolactin levels in the normal (2.5–20 ng/ml; Ref. 17) to stress range (10–100 ng/ml; Ref. 43) are associated with immune preservation, particularly in the setting of an apoptotic stimulus, such as stress cortisol release. In this regard, our findings support the animal data documenting the immune-suppressive effects of hypoprolactinemia in states of stress. Administration of prolactin secretagogues such as metoclopramide and haloperidol to human subjects have been shown to raise prolactin into the stress range (44, 45). These drugs may deserve investigation for use in critically ill populations who are at risk for lymphopenia lymphoid depletion and nosocomial sepsis.

There are limitations to consider in our study. A prolactin-binding protein that interferes with biologic activity is known to exist. Its binding characteristics in pathologic states have not been described, so it is unclear how protein binding might affect the prolactin levels measured in this study (46). The biologic activity of immunoreactive prolactin in this patient population may be confirmed in a future study. Because the two patient cohorts were collected at different times, the possibility that changes in clinical practice over time influenced patient outcomes cannot be ruled out. The TUNEL method used in autopsy specimens only identified the group of cells that were in the process of apoptotic death. Cells that are identifiable as apoptotic are phagocytosed within a few hours (47). Although a delay in tissue fixation has been reported to increase the percentage of cells that nick end labeling in situ, the delay was similar for the groups with and without TUNEL-positive tissues. We documented lymphocyte depletion and apoptosis in patients who died; however, we can only speculate on the causes of prolonged lymphopenia in patients who recovered. Recent publications have reported the presence of early indicators of apoptosis in the circulating lymphocytes of moderately critically ill patients (48). Extrapolation of these findings directly to adults will also require further study. Lymphocyte numbers decrease with age; therefore, the ALC < 1000 cutoff may or may not be predictive in adults. Also, the role of different prolactin secretagogues will likely be important. For example, metoclopramide is used commonly in critically ill children, whereas haloperidol (another central dopamine antagonist) is used commonly in critically ill adults.

In summary, prolonged lymphopenia identifies critically ill infants and children at high risk for nosocomial infection, lymphoid depletion, and death from nosocomial sepsis-induced MOF. Occult hypoprolactinemia likely contributes to the pathogenesis of this condition. What prolactin level is associated with the best outcome from critical illness has yet to be determined. Investigations of novel strategies that 1) substitute for lymphocyte function (e.g., antibiotic prophylaxis against infection for low CD4 counts and IVIG supplementation for hypogammaglobulinemia), 2) improve lymphocyte function (e.g., zinc supplementation), 3) remove iatrogenic sources of lymphopenia (e.g., rapid tapering of steroids, lympholytics), and 4) reverse hypoprolactinemia (e.g., dopamine infusion withdrawal, administration of prolactin secretagogues, or careful titration of prolactin infusion to target levels associated with immune preservation) are warranted in this easily identified critically ill population.

Acknowledgments

We thank Christine Marco and Steve Tomarello for their technical support.

Disclosures

The authors have no financial conflict of interest.

References

Lymphopenia and Lymphocyte Apoptosis and Hypoprolactinemia


