Relationship between serum progesterone and tumor necrosis factor production in postmenopausal women undergoing estrogen/medroxyprogesterone therapy

Changes in tumor necrosis factor (TNF) production by lipopolysaccharide-stimulated peripheral blood mononuclear cells significantly correlated with serum P levels in postmenopausal women subjected to estrogen/medroxyprogesterone therapy. This may suggest that low physiologic or hormone therapy-related changes of serum P in healthy postmenopausal women may affect an ability of peripheral blood mononuclear leukocytes to produce TNF, thus having an impact on a variety of TNF-dependent physiologic and pathologic phenomena. (Fertil Steril® 2009;91:1344–6. ©2009 by American Society for Reproductive Medicine.)

Sex steroids are thought to participate in regulation of immune response and may play a part in modulation of some inflammatory and autoimmune disorders (1, 2). Accordingly, loss of sex steroid production (e.g., after menopause) may affect immune reactivity, including proinflammatory cytokine production, and some of these changes may be partially reversed by hormone therapy (HT) (3–5). However, the mechanisms of HT effects on the immune system seem to be very complex and still remain to be elucidated.

Tumor necrosis factor (TNF), a pluripotent cytokine involved in induction of inflammatory reactions, immunoregulation, connective tissue turnover and degradation, osteoporosis, antitumor responses, and lipid metabolism (6) is one of the cytokines whose activity is postulated to underlie some menopause-associated disorders. Several investigators reported a significant decrease or a trend toward decreased levels of TNF production after HT (7, 8), whereas others reported an increase (9) or no change in TNF production (10). These results suggest that the effect of HT on TNF production still needs verification. Therefore, the aim of the present study was to evaluate production of TNF by lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC) before and after estradiol/medroxyprogesterone therapy.

The Medical University of Warsaw Ethics Committee approved the experimental protocols, and all patients gave informed consent to be included the study. The study included 24 women (median age, 56 years; range, 44–62 years) recruited at the outpatient clinic of the Department of Endocrinology, Postgraduate Medical Center, who completed a 90-day course of HT consisting of three cycles of 0.625 mg/d of conjugated equine estrogens for 28 consecutive days and 5.0 mg/d of medroxyprogesterone acetate from day 19 to 28 with no therapy-free days. Subjects were qualified when they met all of the following criteria: complete cessation of menses for at least 1 year, severe menopausal symptoms (Kupperman index >20), low level of estrogen (<50 pg/mL), high level of FSH (>15 IU/L), no previous history of HT, and lack of any chronic internal systemic disorders (e.g., endocrine, cardiovascular, renal, liver, and/or neoplastic, autoimmune, and inflammatory diseases). Patients were not subjected to any therapy known to affect the immune system for at least 1 year before and during the onset of the study. Individuals with any infectious diseases and/or taking steroids or nonsteroidal anti-inflammatory drugs just before and during the onset of the study were excluded.

For serum and PBMC isolation, 10 mL of peripheral blood was taken from each patient 1 day before and on day 90 ± 3 of the therapy. Serum levels of E_2, P, and FSH were quantitated by specific immunochemiluminescence assays (IMMULITE 2000; DPC, Los Angeles, CA) according the manufacturer's instructions. The PBMC were routinely isolated from heparinized (50 U/mL) peripheral blood by Ficoll-Paque (Pharmacia, Uppsala, Sweden) discontinuous gradient centrifugation, suspended in RPMI 1640 medium supplemented with 10% fetal calf serum and 1% antibiotic–antimycotic solution (all from GIBCO; Paisley, Scotland, United Kingdom), and inoculated onto a 24-well plate (Corning, Corning, NY) at a density of 2×10^6 cells per 1 mL per well. After culture at 37°C in a humidified atmosphere of 5% CO_2 for 24 hours with addition of 1 μg/mL of *Escherichia coli* lipopolysaccharide (LPS; Sigma, St. Louis, MO), cell-free supernatants were harvested and used for determination of TNF. Cell-free supernatants generated by unstimulated PBMC cultured in medium alone served as controls. Tumor necrosis factor was evaluated using a commercial specific Quantikine ELISA kit

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(R&D, Minneapolis, MN) according to the manufacturer's instructions.

In all cases TNF production by unstimulated PBMC was below the limit of detection (data not shown). Median TNF production by LPS-stimulated PBMC at the onset of the therapy was 151.5 pg/mL (range, 29.0–308.0; 95% confidence interval [CI] 125.4–199.0). Median TNF production after 90 days of HT was 178.0 pg/mL (range, 22.0–571.0; 95% CI 140.0–275.8). This difference was not statistically significant (P=.34) as judged by nonparametric Wilcoxon matched-pairs signed-rank test. However, TNF production showed very high variability between individual patients, which might be explained by differences in their genetic background, such as functional TNF gene polymorphism (11). Such a great variance of TNF values considerably decreased the power of the study. Therefore, an effect of estrogen/medroxyprogesterone therapy on TNF production by stimulated PBMC cannot be definitely excluded.

Concentrations of TNF in culture supernatants did not correlate with respective serum levels of E_2, P, or FSH (data not shown). Lack of direct correlation between the levels of TNF and these hormones may also reflect high variability in TNF production. Therefore, to eliminate the effect of this variability, for evaluation purposes we used before/after therapy indices reflecting relative change of TNF and hormone levels between both evaluation points. Spearman rank order correlation analysis revealed a significant negative correlation between TNF and P before/after therapy indices (Fig. 1). A similar correlation between TNF and E_2 or FSH was not observed.

This finding may be supported by in vitro observations that medroxyprogesterone inhibits TNF and other proinflammatory cytokine production by PBMC from cancer patients (12) and that P inhibits TNF expression in murine macrophages (13). It should be noted, however, that a positive relationship between circulating P and spontaneous TNF production by PBMC has been reported in healthy women during the luteal phase of the menstrual cycle (14). Furthermore, other studies did not reveal any direct effect of P on TNF production by PBMC (15), or even showed that P may stimulate TNF release from LPS-stimulated PBMC (16).

In conclusion, results from the present study suggest that low physiologic or HT-related changes of serum P levels in healthy postmenopausal women may affect an ability of peripheral blood mononuclear leukocytes to produce TNF, thus having an important impact on a variety of TNF-dependent physiologic and pathologic phenomena.

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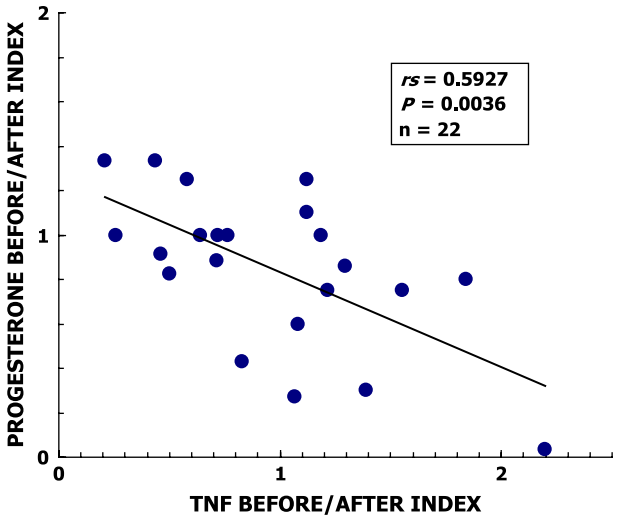
FIGURE 1

Correlation between relative before/after estrogen/medroxyprogesterone replacement therapy differences (before/after index) in TNF production by LPS-stimulated PBMC and serum P levels in postmenopausal women. Each point represents an individual patient. Spearman correlation coefficient (*rs*), its probability level (*P*), and number of presented cases (n) are also shown.



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