Correlations in the Cytokine System in Endometrial Hyperplasia

A. V. Zhdanov, G. T. Sukhikh, M. P. Davydova*, T. V. Slukina, G. E. Chernukha, T. E. Samoilova, and V. P. Smetnik

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Correlations between local expression of insulin-like growth factor 1, insulin-like growth factor receptor, epithelial growth factor, transforming growth β_2 factor, PCNA, TNF-α, TNF receptor 1, Fas, FasL, IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-12 genes in intact and hyperplastic endometrium and in the endometrum after hormone therapy were analyzed. Numerous correlations at the proliferation and secretion stages of the menstrual cycle indicate balanced cytokine system. The number of correlations decreases in glandular cystic and more so in atypical hyperplasia, indicating imbalance in the cytokine system. Dufastone and zoladex therapy did not lead to recovery of this balance, but higher correlations between the expression of some factors of cell proliferation attest to the beginning of normalization of pathologically changed endometrium.

Key Words: *mRNA; cytokines; endometrial hyperplasia; correlation*

Cyclic changes in the endometrium during the menstrual cycle depend on fluctuations in local levels of many factors, primarily hormones and cytokines. The cytokine system is an intricate multicomponent network in which changes in the content of one factor induce a cascade of changes in the expression of cytokine genes, on the one hand, and are to be compensated by production of cytokines equilibrating the system, on the other. Such relationships exist for many cytokines expressed in the endometrium. It is known that IL-8 increases the production of FasL at early stages of the menstrual cycle [10]. Numerous growth factors (PGDF, epithelial growth factor — EGF), oncogenes and oncosuppressors are involved in the regulation of expression of type 1 insulin-like growth factor (IGF-1) and its receptor (IGF-R1) genes [3]. Th-1 type cytokines IFN-γ and TNF-α block the effect of IGF-1 [8]. The production of many chemokines involved into angiogenesis, apoptosis, and proliferation in the endometrium is regulated by Th1 type proinflammatory cytokines TNF-α, IFN-γ, and IL-1 [5]. TNF-α and IL-1 activate factors of NFkB and AP1 transcription, which induce the expression of many cytokines [11].

Endometrial hyperplasia (EHP) involves changes in the expression of cytokine genes. The cascade compensatory relationships are common for the cytokine system, and we studied changes in correlations between the expression of genes for some cytokine, proliferation and apoptosis factors during transformation of normal endometrium into hyperplastic.

MATERIALS AND METHODS

Specimens of the endometrium with glandular cystic (GCH, n=17) and atypical (AH, n=12) hyperplasia were examined. Endometrium specimens from healthy women during proliferation (n=5) and secretion phases (n=5) served as the control. Patients with GCH were treated with dufastone in a daily dose of 20 mg starting from days 5 or 12 until day 25 of the cycle; pa-

Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow; *Faculty of Fundamental Medicine, M. V. Lomonosov Moscow State University

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tients with AH received zoladex (3-6 subcutaneous injections in a dose of 3.6 mg once in 28 days).

Correlations between the expression of the following genes were evaluated: IGF-1, IGF-R, EGF, TGF-β_2, PCNA (proliferation activation), TNF-α, TNF-R1, Fas, FasL, IFN-γ (proliferation suppression or apoptosis activation), IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-12 (regulation of the menstrual cycle and inflammatory processes).

Isolation and DNAse treatment of RNA, reverse transcription, polymerase chain reaction, and quantitative analysis of the data were carried out as described previously [2]. The level of each factor mRNA production at the stage of secretion was taken for one unit. The levels of correlations were estimated using STATISTICA software. The distribution was evaluated using Kolmogorov—Smirnov test, as the distribution of the samples differed from the normal, and correlations were estimated using Spearman's test and were considered significant at $p<0.01$.

RESULTS

Positive correlations between the expression of proliferation factors IGF-1 and PCNA were detected in the control group ($n=10$) ($Rs=0.79$, $p<0.01$). Significant correlations were observed between the levels of expression of TNF-α and some cytokines: proliferative PCNA ($Rs=0.78$, $p<0.01$) and antiproliferative IFN-γ ($Rs=0.83$, $p<0.01$), apoptosis inductor FasL ($Rs=0.9$, $p<0.001$) and its receptor ($Rs=0.99$, $p<0.001$). The level of TNF-R1 receiving the signal to apoptosis positively correlated with the levels of both pro- (Fas, $Rs=0.85$, $p<0.01$) and antiapoptotic factors (IGF-R, $Rs=0.83$, $p<0.01$). Correlations between proapoptotic FasL factor, functionally related to TNF-α, and its receptor and the proliferation factor PCNA ($Rs=0.82$, $p<0.01$, and $Rs=0.94$, $p<0.0001$, respectively) were detected. In addition, the level of FasL gene expression positively correlated with the level of its own receptor ($Rs=0.88$, $p<0.001$) and antiproliferative IFN-γ ($Rs=0.78$, $p<0.01$). Proinflammatory IL-1β positively correlated with pro- and antiproliferative factors: Fas ($Rs=0.7$, $p<0.01$) and PCNA ($Rs=0.77$, $p<0.01$).

The number of correlations was lower in the groups with different types of hyperplasia. Normal correlations between FasL and Fas, FasL and PCNA, Fas and PCNA remained unchanged only in the GCH group ($Rs=0.68$, $p<0.01$; $Rs=0.72$, $p<0.001$, and $Rs=0.65$, $p<0.01$, respectively), while the correlation between TNF-α and Fas ($Rs=0.9$, $p<0.0001$) remained significant only in atypical hyperplasia. Normally absent significant correlation between Fas and IGF-R ($Rs=0.81$, $p<0.001$ for GCH and $Rs=0.9$, $p<0.0001$ for AH) was detected in both groups of patients. Some correlations not detected in health, but developing only in one form of hyperplasia were detected: between IGF-R and IGF, IGF-R and PCNA in GCH ($Rs=0.67$, $p<0.01$ and $Rs=0.72$, $p<0.001$, respectively) and between FasL and IFN-γ in AH ($Rs=0.73$, $p<0.001$).

The number of correlations in patients decreased after therapy. Correlation between Fas and IGF-R, found in both types of hyperplasia, was not detected after the treatment. By contrast, the level of PCNA mRNA still correlated with IGF-R in the GCH group after dufastone therapy ($Rs=0.94$, $p<0.01$) and Fas correlated with IGF-R in AH group after zoladex therapy ($Rs=0.94$, $p<0.01$). New correlations, not characteristic of the hyperplasia groups and controls, appeared after therapy. The level of Fas mRNA correlated with the expression of IGF-1 gene ($Rs=0.94$, $p<0.01$) after dufastone therapy, while zoladex treatment led to the appearance of significant associations between TNF-R1 and IL-1β ($Rs=0.94$, $p<0.01$) and between TNF-R1 and IGF-R ($Rs=-0.94$, $p<0.01$). A correlation absent in hyperplasia, but present normally appeared: between PCNA and IGF-1 ($Rs=0.94$, $p<0.01$ for both groups).

Correlations can result from either simultaneous activation of cytokine expression by one factor or regulation of one cytokine expression by another cytokine, or compensatory modulation of the expression of one gene in response to modulated expression of some other genes. The cytokine system can be regarded as a network in which the increase or decrease in the production of any component induces changes in the type of expression of factors coupled to it. This involves destruction of many existing correlations, after which new correlations develop, if the cytokine system attains balance again. Analysis of our findings indicates that the cytokine system in intact endometrium is balanced, which is seen from correlations between many cytokines, proliferation and apoptosis factors, while in endometrial hyperplasia and after its therapy with hormones the balance was essentially distorted.

We observed two associated groups of factors in AH. On the one hand, expression of apoptosis-inducing Fas receptor was associated with the expression of IGF-R, its functional antagonist [9], and with expression of TNF-α, which can activate apoptosis mediated by Fas-FasL interactions [7]. On the other hand, the expressions of FasL correlated with expression of antiproliferative IFN-γ. In glandular cystic hyperplasia expression of Fas, FasL (apoptosis inductors) and PCNA (proliferation activator) correlated, the expression of both PCNA and Fas genes correlated with the production of IGF-R mRNA, while the latter, in turn, was closely associated with expression of its ligand IGF.

In general, AH is associated with a lesser number of correlations and the absence of correlations between

the content of mRNA of proteins directly reacting with each other (for example, between IGF-1 and IGF-R, Fas and FasL). Correlations between proapoptotic factors were more incident. However, according to published reports, apoptosis in endometrial hyperplasia is just slightly more intense in comparison with the proliferation stage [6]. Presumably, programmed cell death in hyperplasia is inhibited at another regulatory level, specifically, due to reduced activity of the nuclear Ca^{2+}/Mg^{2+}-dependent endonucleases [1].

Cell proliferation in endometrial hyperplasia was also suppressed, which reflected the absence of the majority of correlations with PCNA (particularly in AH), characteristic of the normal endometrium. Our findings are in line with the results of morphological studies, according to which the number of mitoses in hyperplasia is lower than during the proliferation stage [4].

Decreased number of correlations between apoptosis and proliferation factors after therapy indicates that hormone treatment distorts the balance in the cytokine system, which is seen from changed expression of the cytokine genes (trend to normalization or still more marked deviations from normal). However, enhanced production of proliferation activators PCNA and IGF-1 mRNA and recovery of the correlation between them, as well as decreased expression of proapoptotic factor Fas indicate the creation of molecular prerequisites for transition of the hyperplastic endometrium into a state of normal proliferation.

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