Immunomorphological Characteristics of Endometrium in Women with Chronic Endometritis

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The number of monocytes, macrophages ($CD14^+$), and large granular lymphocytes ($CD56^+$) in the endometrium increased in chronic endometritis. These changes are an unfavorable prognositic signs for the onset of pregnancy. Combination therapy improved the phenotypic composition of endometrial cells.

Key Words: *endometrium; chronic endometritis; immunomorphology*

The endometrium contains a considerable number of immunocompetent cells. The phenotypic composition of these cells plays an important role in the maintenance of the immune balance between the embryo and endometrium and determines implantation and placentation [3,5,7]. Large granular lymphocytes (LGL) are the most prevalent population of endometrial leukocytes: during the proliferative and secretory phases of the cycle and at early terms of pregnancy they constitute 8, 60, and >70% of all endometrial cells, respectively [1,3]. LGL, T cells, and macrophages of the endometrium are the main source of cytokines that provide the dominance of a Th-2 immune response during pregnancy. Changes in the number of LGL in the endometrium during bacterial or viral infections and inflammation disturb the cytokine balance (Th-1 dominance). These changes result in inhibition of trophoblast invasion and miscarriage [4,5]. Variations in the ratio between subpopulations and activity of cells during chronic endometritis can trigger cascade pathological reactions that lead to sterility or miscarriage [2,6].

Here we studied the phenotypic composition of endometrial cells in women with chronic endometritis. The effect of therapy on the test parameters was estimated.

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MATERIALS AND METHODS

We examined 45 women (mean age 27.4±3.3 years) with chronic endometritis and reproductive dysfunction, which included sterility, miscarriage, and unsuccessful extracorporeal fertilization (ECF). The diagnosis was verified by the results of diagnostic curettage (proliferative phase, days 7-10 of the menstrual cycle) followed by histological and immunomorphological examination of endometrial samples. Therapy of patients with chronic endometritis included treatment with etiotropic (ofloxacin, Wilprafen, Augmentin, Tiberal, *etc*.), antiinflammatory, spasmolytic, immunomodulatory, and metabolic drugs and physiotherapeutic procedures. The duration of therapy was 4 months. The endometrium was repeatedly examined on days 7-10 of the cycle (2 months after the end of therapy).

The control group included 12 healthy women of reproductive age. These women did not use hormonal contraceptives and had no chronic somatic disease or reproductive dysfunction.

For immunophenotyping of endometrial cells, the tissue was minced and filtered through a nylon sieve into medium 199. The isolated cells were re-

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suspended to single cells suspension. The suspension was layered on a Ficoll-Verografin gradient (ρ=1.077) and centrifuged at 800*g* for 20 min to remove erythrocytes. Interphase cells were washed with phosphate buffered saline, diluted to a concentration of 10^6 cells/ml, and used for phenotyping. Endometrial cells were phenotyped on a WinBrite flow cytofluorometer (Bio-Rad). Fluorescent staining involved monoclonal antibodies against CD cell markers conjugated with fluorescein isothiocyanate or phycoerythrin (Caltage). Experiments were performed with CD3 (T lymphocytes), CD4 (T helpers), CD8 (T suppressors), CD14 (monocytes/macrophages), CD16 (natural killer cells, NK), CD45 (leukocytes), CD56 (NK), LGL, and CD95 (Fas antigen).

The cell suspension (100 μl, 10^5 cells) was incubated with antibodies (10 μl) at 4°C for 30 min. The cells were centrifuged in phosphate buffered saline. Cytofluorometric study of the cell suspension was performed in the range corresponding to light scattering of lymphoid cells.

RESULTS

Chronic endometritis was accompanied by a complex of well-defined immunomorphological changes in the endometrium. During the proliferative phase (days 7-10 of the cycle) the number of endometrial monocytes/macrophages ($CD14^+$) and LGL ($CD56^+$) in patients with chronic endometritis was higher than in women of the control group ($p<0.05$, Table 1). We revealed a slight increase in the total number of T lymphocytes ($CD3^+$). The number and ratio between T helpers ($CD4^+$) and T suppressors ($CD8^+$) did not differ in patients with chronic endometritis and healthy women. An increase in the number of LGL and macrophages in the endometrium of women with reproductive dysfunction reflects the severity of tissue inflammation. Published data show that these changes prevent normal adhesion and implantation of the blastocyst and trophoblast development [6,7]. The number of $CD95^+$ cells (Fas receptor) in patients was higher than in healthy women. Therefore, chronic inflammation is accompanied by high-intensity apoptosis.

Morphological characteristics of the endometrium retuned to normal by the end of therapy for chronic endometritis. It was manifested in the absence of plasma cells, lymphocytic infiltration, and edema or fibrosis of the stroma. We revealed a significant decrease in the number of macrophages ($CD14^+$, from 12.7±2.7 to 6.13±1.60%, $p<0.05$) and LGL ($CD56^+$, from 47.1±6.4 to 26.80±8.21%, $p<0.05$, Table 2). These changes are associated with favorable prognosis for pregnancy onset in women with reproductive dysfunction. A slight decrease in the total number of leukocytes ($CD45^+$) and Fas antigen-positive cells ($CD95^+$) reflects the reduction of inflammation and inhibition of apoptosis.

TABLE 1. Phenotypic Characteristics of Endometrial Leukocytes in Women with Chronic Endometritis (*M*±*m*, % of the total number of leukocytes)

Differentiation cluster	Control group	Chronic endometritis
CD14	1.5±0.6	12.7±2.7*
CD3	48.2±10.6	59±3.7
CD4	20.1±6.4	31.4±2.78
CD8	26.8±7.9	27.9±3.6
CD16	5.7±1.5	4.35±0.99
CD56	22.8±7.3	47.1±6.4*
CD95	25.3±4.6	36.7±4.7

Note. *$p<0.05$ compared to the control.

TABLE 2. Phenotypic Characteristics of Endometrial Leukocytes during Therapy of Chronic Endometritis (*M*±*m*)

Differentiation cluster	Before therapy	After therapy	*p*
CD14	12.7±2.7	6.13±1.6	0.01
CD3	59.0±3.7	63.50±3.86	0.2
CD4	31.4±2.78	33.2±4.5	0.47
CD8	27.9±3.6	26.7±1.4	0.36
CD16	4.35±0.99	6.65±1.16	0.35
CD56	47.1±6.4	26.80±8.21	0.04
CD95	36.7±4.7	29.5±4.1	0.31

Chronic inflammation considerably modifies functional potential of the tissue. Taking into account a complex structure and ability to undergo cyclic transformation, pathological changes in the endometrium are particularly pronounced and hardly corrected. Receptivity of the endometrium is determined by a variety of factors. Each of these factors should be analyzed individually. Pathogenetic therapy of women with chronic endometritis and reproductive dysfunction improves structural characteristics and functional activity of the endometrium and counteracts the effect of factors that prevent the onset and normal course of pregnancy.

REFERENCES

1. J. N. Bulmer, *Ann. NY Acad. Sci*., **30**, 185-192 (1994).
2. B. Disep, B. A. Innes, and H. R. Cochrane, *Histopathology*, **45**, No. 6, 625-632 (2002).

3. S. R. Glasser, J. D. Aplin, L. C. Guidice, and S. Tabibzadeh, *The Endometrium*, London, New York (2002), p. 675.
4. J. S. Krasnow, D. J. Tollerud, G. Naus, and J. A. DeLoia, *Hum. Reprod.*, **11**, 1747-1754 (1996).
5. Y. W. Loke and A. King, *J. Reprod. Fertil. Suppl.*, **55**, 83-90 (2000).
6. L. A. Salamonsen, J. Zhang, and M. Brasted, *J. Reprod. Immunol.*, **57**, No. 2, 95-108 (2002).
7. A. M. Sharkey and S. K. Smith, *Best Pract. Res. Clin. Obstet. Gynaecol.*, **17**, No. 2, 289-307 (2003).