Local Production of Interleukins and Growth Factors in External Genital Endometriosis

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Secretion of some IL and growth factors (VEGF, IGF-I, TGFβ) by endometrial tissues and endometrioid heterotopies was studied *in vitro* in patients with external genital endometriosis of different severity. The production of IL-1β, IL-2, IL-6, and VEGF in the endometrium increased in severe external genital endometriosis, while the secretion of TGFβ decreased; hyperproduction of IL-2, IL-6, VEGF and decreased production of TGFβ were detected in endometrioid foci. Presumably, local cytokine imbalance and increased proliferative activity of endometrial cells are involved in the mechanisms of formation and functioning of endometrioid foci.

Key Words: *endometriosis; eutopic and ectopic endometrium; organotypic culturing; interleukins; growth factors*

Recent studies confirmed the involvement of the immune system in the development of external genital endometriosis (EGE). The disease is associated with redistribution of lymphocyte subpopulations (increased percentage of activated and poorly differentiated T cells in parallel with activation of B cell system) [1]. The cytotoxicity of NK cells, induced by the production of IFN-α, IFN-β, and IFN-γ by peripheral blood leukocytes, decreased in EGE [8].

However, despite obvious significance of systemic changes in the immunity in EGE, the processes developing directly in the pelvic peritoneum and leading to the disease progress or to regression of endometrioid heterotopies, play the key role in the disease development. After implantation of endometrial cells to the peritoneal surface further growth of endometrioid tissue is induced by cytokines and growth factors, produced by macrophages of the peritoneal fluid, endometrioid heterotopies, and other adjacent cells [3].

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About 50 cytokines determine cell interactions and modulate their functions in certain pathological processes. These mediators realize their activity mainly through autocrine and paracrine routes by forming a universal regulatory system [9]. The immunoregulatory effects of the cytokine network are based on the balance of opposite molecular pools, while their imbalance leads to the development of pathological states [10]. Presumably, changes in the ratio of pro- to antiinflammatory cytokines creates predisposition for the development of EGE.

We studied secretion of some IL and growth factors by endometrial tissue and endometrioid foci under conditions of organotypic culture.

MATERIALS AND METHODS

Forty-three women with EGE aged 21-39 years (mean age 29.4±4.3 years) were examined. The diagnosis was verified by endoscopic data and results of histological analysis of the operation material. Stage I-II EGE was diagnosed in 27 and stage III-IV EGE in 16 patients (in accordance with classification of American Fertility Society, R-AFS).

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The control group consisted of 20 healthy women of reproductive age hospitalized for surgical sterilization.

Material for the analysis was collected during laparoscopic interventions. Tissue explants from eutopic endometrium and endometrioid heterotopies were cultured *in vitro* in 4-well plates (Nunc) in 1 ml RPMI-1640 with 10% FCS (Biocell) for 24 h at 37°C and 5% CO_2 in a Sanyo incubator. After incubation the supernatant was collected and frozen in sterile polypropylene tubes at -20°C. The explants were dried on filter paper and weighed on analytical balance for standardization of the initial biological material.

Cytokines and growth factors were measured in the culture medium by enzyme-linked immunosorbent assay (ELISA).

The production of IL-1β, IL-2, and IL-6 was evaluated using Protein Contour test system, production of vasculoendothelial growth factor (VEGF) by test system manufactured by Cytoimmune Sciences Inc., production of transforming growth factor-β1 (IGFβ1; Biosource internation), and production of insulin-like growth factor-1 (IGF-1) by a kit manufactured by Diagnostic System Laboratories Inc. The results were recorded using Multiscan MCC 344 microplate photometer (Labsystems).

The results are presented as the mean levels of cytokines secreted into the culture medium per mg wet tissue. The significance of differences was evaluated using Student's *t* test.

RESULTS

The maximum level of IL-1β in cultures of eutopic endometrium was observed in disseminated EGE (stage III-IV); this level surpassed the values in the control group and in mild forms (stage I-II) of the disease (Table 1).

The production of IL-2 by endometrial explants was maximum in severe EGE (14-fold higher than in the control; Table 1).

Secretion of IL-6 by endometrial tissue of patients with EGE far surpassed the control level and was maximum in disseminated forms of the disease (Table 1).

Evaluation of growth factor secretion by endometrial tissues of patients with EGE showed the highest concentration of VEGF in culture supernatants in severe EGE forms. The production of this factor increased by 42 and 75% in comparison with the control group and patients with stage I-II EGE, respectively (Fig. 1).

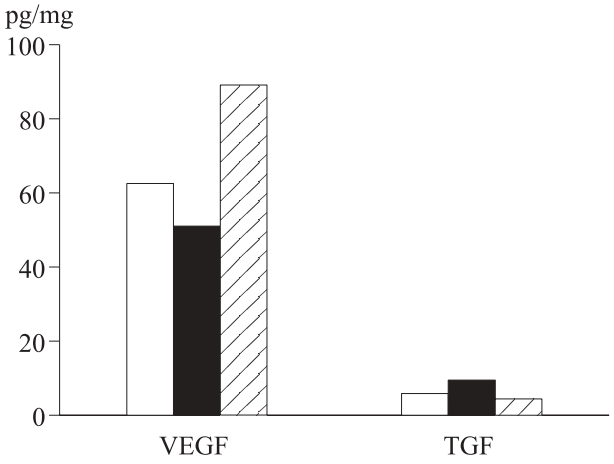

Fig. 1. Secretion of VEGF and TGFβ by endometrial explants. Here and in Figs. 2 and 3: light bars: control; dark bars: external genital endometriosis (EGE) of stage I-II; cross-hatched bars: stage III-IV EGE.

The production of TGFβ in the endometrium in severe disease was 2-fold lower than in stage I-II EGE (Fig. 1).

The concentrations of IGF-1 in supernatants of eutopic endometrium from EGE patients and healthy women did not differ significantly, but production of this growth factor tended to increase in progressive disease (Fig. 2).

The intensity of IL-2 and IL-6 secretion in endometrioid foci in severe EGE forms was approximately 3-fold higher than in stage I-II EGE (Table 2).

The production of VEGF in severe EGE was 80% higher than in mild forms (Fig. 3).

The secretion of TGFβ in endometrioid foci decreased in disseminated forms of EGE, similarly as during culturing of eutopic endometrium (Fig. 3), while production of IGF-1 remained virtually unchanged in EGE of different severity (Fig. 2).

Specific features of EGE development allowed us regard this disease as a tumor-like process. Activity of

TABLE 1. Secretion of IL by Endometrial Explants from EGE Patients and Healthy Women (*M*±*m*)

Cytokines, pg/mg tissue	Control group (*n*=20)	EGE	
		stage I-II (*n*=27)	stage III-IV (*n*=12)
IL-1β	4.3±1.1	3.4±0.9	5.8±2.5*+
IL-2	0.6±0.5	5.7±2.2	8.6±3.1*
IL-6	180.7±58.1	235.8±91.5*	546.7±207.8*

Note. $p<0.05$ compared to *control group; +EGE of stage I-II.

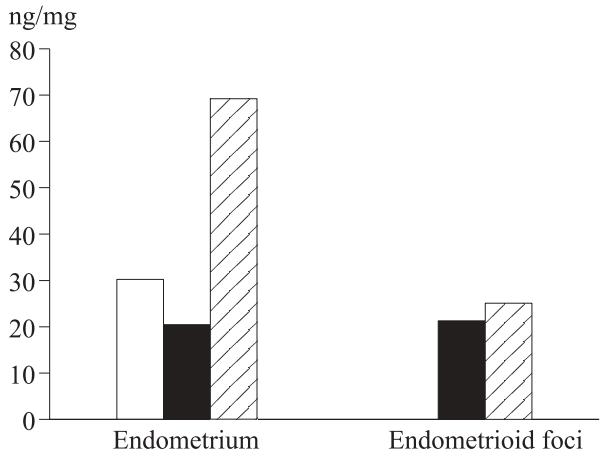

Fig. 2. Secretion of IGF-1 by explants from eutopic endometrium and endometrioid foci.

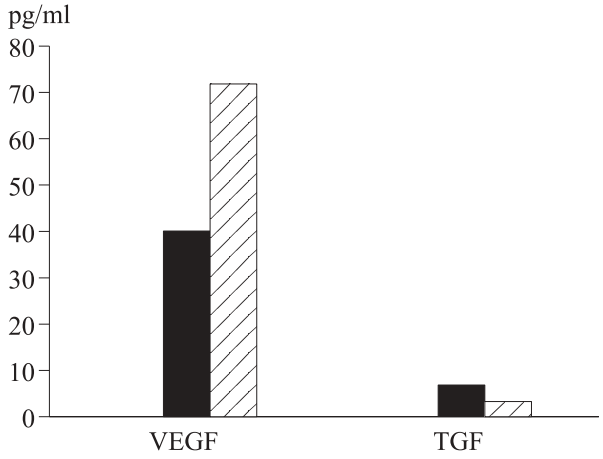

Fig. 3. Secretion of VEGF and TGFβ by explants from endometrioid foci.

proliferation in endometrioid foci largely depends on the presence of certain cytokines, their ratio, intensity of secretion, and local concentrations. Evaluation of these parameters for the whole body is difficult because of predominantly local action of these cytokines, but this can be done *in vitro*. Culture models do not completely reproduce *in vivo* processes, but give an idea of their regularities in health and disease and allowed evaluation of some quantitative characteristics of local production of some cytokines.

TABLE 2. Secretion of IL by Explants from Endometrioid Foci of Patients with EGE (*M*±*m*)

Cytokines, pg/mg tissue	EGE, stage I-II (*n*=26)	EGE, stage III-IV (*n*=15)
IL-1β	17.1±15.6	2.2±1.3
IL-2	2.4±0.6	7.0±1.9*
IL-6	204.3±54.3	649.3±106.2*

Note. *$p<0.05$ compared to stage I-II EGE.

Our experiments demonstrated decreased production of proinflammatory cytokine IL-1β by endometrioid focus cells in severe endometriosis. The production of IL-6 increased; this protumorigenic and antiinflammatory cytokine is opposite to IL-1β [4]. Increased IL-6 content in the endometrioid focus can intensify proliferative processes. Increased production of IL-2 in stage III-IV EGE probably reflects the compensatory reaction of the immune system, aimed at stimulation of cytotoxic cells and of antitumor activity [7].

An obligatory condition for the existence and function of tumor implants is their neovascularization [13]. VEGF stimulating endotheliocyte proliferation and involved in the regulation of vascular permeability is a key regulator of angiogenesis [12]. Intensification of VEGF secretion by cultures of endometrioid heterotopies with increasing EGE severity suggests that increased secretion of this factor in the endometrioid focus *in vivo* promotes the development of subperitoneal vascular network with the disease progress. Expression of VEGF gene in macrophages can be induced by IL-6 [11], which determines a direct correlation between the levels of these cytokines.

Decreased secretion of polyfunctional cytokine TGFβ can serve as a factor promoting the development of endometrioid foci. This factor causes numerous biological effects [2], including antiinflammatory activity and suppression of invasive growth of cells and tissues [5,10].

Analysis of our findings suggests that intensification of angiogenesis in tumor implants can be caused by, among other things, suppression of TGFβ production inhibiting angiogenesis in EGE.

Synchronous increase in the production of proinflammatory cytokine IL-1β and protumorigenic mediator IL-6 presumably determines the capacity of eutopic endometrium to implantation in EGE patients. The detected increase in IL-1β, IL-2, and IL-6 production is in line with published data on high incidence of combination of inflammatory changes in the endometrium with EGE [6].

The increase in VEGF secretion paralleled by decreased production of TGFβ in the eutopic endometrium in severe EGE forms seems to be responsible for high angiogenic potential of endometrial cells in patients with EGE, promoting their implantation and growth in the peritoneal cavity.

Presumably, local cytokine imbalance and increased proliferation capacity of endometrial cells play an important role in the mechanisms of formation and functioning of endometriosis foci.

On the whole, the results of our *in vitro* study are in line with modern concepts of the pathogenesis of endometriosis: increased local production of factors stimulating angiogenesis and cell proliferation and

suppression of inhibitory factors. Presumably, changes/disorders in the production of a limited number of cytokines are primary; these changes trigger cascade shifts in local cytokine balance (a characteristic mechanism of cytokine effect). Further studies will determine and specify these key factors in EGE pathogenesis. This task is difficult, but necessary for developing the strategy of effective treatment of this disease.

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