IMMUNOLOGY AND MICROBIOLOGY

Cytokine Production by Immunocompetent Cells of Peritoneal Fluid in Women with External Genital Endometriosis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 6, pp. 646-649, June, 2004
Original article submitted January 16, 2004

The content of some cytokines in the peritoneal fluid and the level of their *in vitro* production by peritoneal macrophages and lymphocytes were evaluated in women with external endometriosis. The ratio of peritoneal lymphocytes and macrophages in the peritoneal fluid was changed in endometriosis because of increased percentage of lymphoid cells and decreased content of macrophages, in the presence of high absolute counts of both types of cells. The cytokine status of women with endometriosis was characterized by higher levels of IL-1β, TNF-α, and epidermal growth factors both in the peritoneal fluid and supernatants of 24-h cultures of peritoneal macrophages; the content of IFN-α remained unchanged. The concentrations of IFN-γ in the peritoneal fluid did not change in endometriosis, but increased in the supernatants of peritoneal lymphocyte cultures.

Key Words: *cytokines; peritoneal fluid; endometriosis*

Endometriosis is one of the most prevalent gynecological diseases. According to different authors [1] its incidence varies from 5-50%. However, despite long-term studies the pathogenesis of endometriosis remains not quite clear. It is considered that disorders in local immune response are among the priority factors contributing to survival and implantation of viable fragments of the endometrium in the peritoneal cavity [8]. Great recent attention was paid to studies of the content of various cytokines and growth factors in the peritoneal fluid (PF) [6,13,14], but there are different opinions about the cytokine profiles of PF in women with external endometriosis.

The majority of cytokines detected in PF are of the macrophageal origin. It was shown that the secretion of IL-1β, TNF-α [11], IL-6 [10], IL-10 [15] by peritoneal macrophages increased in endometriosis. The majority of scientists support the hypothesis about hyperactivation of peritoneal macrophages in endometriosis, which results in changes in the cytokine content in patients with PF. However, the data on the cytokine levels are sometimes contradictory, and activation of peritoneal macrophages does not lead to elimination of endometriosis foci in the peritoneal cavity; this leaves the problem on the precise mechanisms of macrophage involvement in the regulation of local cytokine production in endometriosis unsolved.

We analyzed the levels of some cytokines in PF, their *in vitro* production in women with external endometriosis, and evaluated the degree of involvement of some immunocompetent cells into changes in the local cytokine status in this disease.

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0007-4888/04/1376-0568

MATERIALS AND METHODS

Peritoneal fluid specimens from 60 women were analyzed. External endometriosis of different severity according to R-AFS classification was diagnosed at laparoscopy in 32 women suffering from sterility (I-II degree endometriosis in 20 of these and III-IV degree condition in 12). The endocrine causes of sterility, "cervical", tubal, and male factors were excluded. Control group consisted of 28 healthy fertile women hospitalized for surgical sterilization.

PF was collected during laparoscopy. Cytokines were evaluated in the supernatant fraction after centrifugation of PF. The supernatant fraction was frozen and stored at -20°C in sterile flasks. The volume of PF and the absolute and relative counts of lymphocytes and macrophages were measured before freezing.

Cytokine production by peritoneal immunocompetent cells was evaluated by the cytokine content in supernatants (SN) of short-living cultures of peritoneal lymphocytes and macrophages. Peritoneal lymphocyte- and macrophage-rich populations were prepared by PF centrifugation in the Ficoll-verograffin density gradient (d=1.078) and subsequent standard 2-step separation of the mixture of peritoneal mononuclear leukocytes into fractions adhering and not adhering to the plastic. Nonadhesive fraction of peritoneal cells contained 85-90% lymphocytes, in the adhesive fraction the percentage of macrophages was 87-90% of all cells. Cell viability evaluated by trypan blue exclusion was at least 95%. The resultant fractions with high content of peritoneal lymphocytes and macrophages were incubated in RPMI-1640 with 2 M glutamine at cell concentration of 2×10^6 cell/ml at 37°C and 5% CO_2. After 24 h SN was removed, centrifuged at 6000 rpm, and sterilized by filtration through Millipore filters (0.22 μ pores) (Synpor). SN of PF lymphocyte and macrophage cultures derived from fertile women and women with endometriosis were pooled (at least 10 individual SN per pooled sample) and stored in sterile flasks at -20°C.

The concentrations of IL-1β, TNF-α, IFN-γ, and IFN-α were measured by enzyme immunoassay on a Multiscan EX microplate reader (Labsystems). Test systems with threshold sensitivity of 15 pg/ml (ICN) were used for evaluation of IL-1β and TNF-α, test systems with threshold sensitivity of 19 pg/ml (Tsytokin Firm) were used for evaluation of IFN-γ and IFN-α. The concentration of epidermal growth factor (EGF) was measured by competitive enzyme immunoassay using ACCUCYTE kit (Cytimmune), the threshold sensitivity of the test system being 0.195 ng/ml.

The data were statistically processed with evaluation of $M\pm m$. The significance of differences was evaluated using Student's t test.

RESULTS

The volume of PF and the content of mononuclear leukocytes in it were virtually the same in patients with endometriosis and healthy fertile women (Table 1). The absolute and relative counts of lymphocytes increased significantly in PF of endometriosis patients ($p<0.05$ and $p<0.01$, respectively). A marked increase in the absolute count of macrophages in PF of endometriosis patients was paralleled by a significant decrease of their relative content ($p>0.05$ and $p<0.05$, respectively).

The levels of TNF-α, IL-1β, and EGF in PF drastically increased in endometriosis ($p<0.001$, $p<0.01$, and $p<0.01$, respectively; Table 2). The differences in the concentrations of IFN-γ and IFN-α in PF of patients with endometriosis and controls were statistically negligible ($p>0.05$).

Analysis of the cytokine content in SN of 24-h cultures showed that the production of IL-1β and TNF-α by peritoneal macrophages increased in pa-

TABLE 1. Comparative Characteristics of Absolute and Relative Content of Mononuclear Cells in PF of Healthy Fertile Women and Women with External Endometriosis ($M\pm m$)

Parameter	Control	Endometriosis
PF volume, ml	10.4±3.9	10.4±5.1
Concentration of mononuclear cells, $\times10^3$ cell/ml	1.5±0.7	2.8±1.1
Absolute count of mononuclear cells, $\times10^6$ cell	15.4±5.9	22.9±10.3
Lymphocyte content		
relative, %	19.4±4.3	33.5±3.1*
absolute, $\times10^6$ cell	2.8±1.3	9.2±2.3**
Macrophage content		
relative, %	80.5±4.2	65.5±5.0**
absolute, $\times10^6$ cell	12.3±4.1	19.3±5.8

Note. *$p<0.01$, **$p<0.05$ compared to the control.

TABLE 2. Cytokine Content in PF of Women with External Endometriosis (*M*±*m*)

Parameter	Control (*n*=28)	Endometriosis (*n*=32)
TNF-α, pg/ml	<15.0	103.5±11.3*
IL-1β, ng/ml	0.30±0.05	26.40±8.7**
IFN-γ, pg/ml	919.06±361.26	740.65±134.15
IFN-α, pg/ml	89.03±20.22	114.46±33.92
EGF, ng/ml	4.17±0.26	5.79±0.46**

Note. *$p<0.001$, **$p<0.01$ compared to the control.

tients with external endometriosis ($p<0.001$, $p<0.05$; Table 3). The content of IFN-α in peritoneal macrophage culture SN from women with endometriosis did not differ from that in controls ($p<0.05$). The difference in the production of EGF by macrophages in different groups was also statistically negligible, though there was a pronounced trend to an increase of EGF content in the macrophage culture SN. On the other hand, IFN-γ level in SN of 24-h cultures of peritoneal lymphocytes appreciably increased in endometriosis ($p<0.01$).

Our findings on increased content of macrophages in PF of women with external endometriosis are in line with the results of other authors [5]. Our data indicate that the ratio of macrophagic to lymphoid cells is shifted in endometriosis.

The increase of the absolute count of peritoneal macrophages was paralleled by increased production of proinflammatory cytokines (IL-1β and TNF-α) and EGF by these cells and an increase in the concentrations of these cytokines in PF, which confirms the hypothesis about macrophage activation [5]. Many studies demonstrated that the content of other cytokines of macrophageal origin, such as IL-6, IL-8, MCP, VEGF, also increased in PF of women with endometriosis [3,6,9,11,13]. Based on these results, a hypothesis about hyperactivation of peritoneal macrophages in PF was put forward. Some authors claim that macrophagal production of various factors promoting cell proliferation and growth increased in endometriosis, which is pathogenetically significant for the development of endometrioid heterotopies [5]. However, the increase of the local level of proinflammatory cytokines and growth factors was not paralleled by changes in the content of IFN-α, an important proinflammatory cytokine produced by macrophages, in PF of women with endometriosis. The level of its production in cell culture also did not change. It was previously shown that IFN-α is characterized by pronounced antiproliferative activity in endometrial cells [12]. Presumably, the absence of changes in its production by peritoneal macrophages is a factor maintaining viability of endometrioid implants in the peritoneal cavity. It is unclear which mechanisms regulate the production of IFN-α and why its production is not stimulated in endometriosis. It seems that the imbalance in macrophagal production of factors stimulating and inhibiting cell growth, as a result of which the content of these factors decreases, is pathogenetically more important for endometriosis development than total hyperactivity of macrophages. Presumably, lymphocytes play an important role in these processes.

TABLE 3. Cytokine Content in SN of 24-h Cultures of Peritoneal Macrophages and Lymphocytes Derived from Women with External Endometriosis (*M*±*m*)

Parameter	Control (*n*=15)	Endometriosis (*n*=23)
TNF-α, pg/ml	147.00±24.84	213.40±18.15**
IL-1β, ng/ml	0.53±0.01	7.24±1.47*
IFN-γ, pg/ml	61.27±1.59	135.61±32.09**
IFN-α, pg/ml	61.90±1.49	69.67±9.57
EGF, ng/ml	2.72±0.63	3.72±0.7

Note. *$p<0.001$, **$p<0.05$ compared to the control.

We revealed an appreciable increase of IFN-γ production by lymphocytes in tissue culture. The concentration of IFN-γ in PF did not change much, which can be explained by reception of this cytokine by target cells, *e. g.*, by macrophages. Other authors also noted the absence of changes in the content of IFN-γ in PF [10]. We failed to find published reports about the production of IFN-γ in peritoneal lymphocyte culture. The detected increase of IFN-γ concentration in SN attests to an increase in the secretory activity of peritoneal lymphocytes in women with external endometriosis, which can be explained by the absence of modified autologous endometrial cells. IFN-γ is produced mainly by type I T-helpers. It seems that in endometriosis this subpopulation of T-helpers is activated. Previous data on the function of lymphoid cells in PF in endometriosis are ambiguous. It was shown that in healthy women peritoneal lymphocytes are represented mainly by type I T-helpers, which produce more IFN-γ and less IL-4 and IL-5, but at early stages of endometriosis the content of activated lymphocytes with $CD3^+HLA\text{-}DR^+$ phenotype was decreased in PF [7]. However some authors observed an increase in the content of $CD3^+HLA\text{-}DR^+$ lymphocytes in PF of women with endometriosis of different severity [2,4]. Increased production of IFN-γ can stimulate functional activity of peritoneal macrophages and production of some proinflammatory cytokines.

Intricate relationships and mutual effects of immunocompetent cells, inducing the cytokine cascade

at the local level in external endometriosis, deserve further investigation. It seems that regulation of their functional activity is impaired in endometriosis, which determines changes in the cytokine profile.

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