MICROBIOLOGY AND IMMUNOLOGY

Location and Intensity of IL-8 and TGF-β_2 mRNA Production in the Fimbrial Compartment of Fallopian Tubes and IL-10 in the Endometrium in Patients with Pyoinflammatory Adnexal Diseases

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The location and level of IL-8 and TGF-β_2 expression in the fimbrial compartment of fallopian tubes and IL-10 expression in the endometrium of women with pyoinflammatory adnexal diseases were studied by *in situ* hybridization. These diseases are associated with considerable changes in the levels of local production of these cytokines. Inflammatory infiltration and epithelial cells were most active producers of IL-9 and TGF-β_2 in the fimbrial compartment of fallopian tubes, while in the endometrium IL-10 gene was expressed at a high level primarily in the glandular epithelial cells.

Key Words: *inflammation of fallopian tubes; cytokines; endometrium; fimbrial compartment; in situ hybridization*

Disorders of the female reproductive function are largely determined by the severity of inflammation in the genital tract, in particular pyoinflammatory adnexal diseases (PIAD).

Acute and chronic inflammations in the abdominal cavity are usually associated with pronounced changes in the functional activity of the systemic and local immunity [7,9,10,12,13] and with migration of cells forming inflammatory infiltration [8,14] directly to the focus of inflammation and activation of immunocompetent cells.

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The induction and progress of inflammatory reaction is realized by cell-cell interactions in which cytokines play the key role. We previously showed that PIAD are associated with increased expression of IL-4, IL-6, IL-8, IL-10, IL-12, and TGF-β_2 genes in the fimbrial compartment of fallopian tubes (FCFT) and of IL-10 gene expression in the endometrium [1]. These changes are paralleled by decreased production of IL-4, IL-8, IL-10, and IL-15 mRNA in the peripheral blood mononuclear cells, while the expression of IFN-γ gene increased [2]. According to our findings, changes in the production of IL-8 and TGF-β_2 in FCFT and of IL-10 in the endometrium were the most pronounced.

The aim of the present study was to confirm our previous results by *in situ* hybridization and to identify the type and location of cells producing these cytokines in PIAD.

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MATERIALS AND METHODS

Analysis of local expression of cytokine genes in FCFT and endometrium by *in situ* hybridization was carried out in 8 patients with PIAD (3 patients with tubo-ovarian formations and 5 with pyosalpinx, mean age 33.5±4.3 years). Control group consisted of 5 patients with uterine myoma (mean age 36.4±4.2 years). Extirpation of the uterus with or without tubes, supravaginal amputation of the uterus with or without tubes, or salpingectomy were carried out according to clinical indications. Material for analysis (tissue fragments, 100-150 mg) was collected in the middle of the proliferative stage of the menstrual cycle.

The material was fixed in Lilly solution for 4 weeks and embedded in histoplast (Shandon) after accelerated histological treatment [3].

In situ hybridization was carried out by the method described previously [4] with modifications. IL-8, IL-10, and TGF-β_2 cDNA amplification products were cloned in pGEM-T plasmid. Antisense and sense probes were obtained using DIG RNA Labeling Kit (SP6/T7) (Roche) as described in the instruction. The probes were stored at -20°C.

Tissue sections (5 μ) were fixed on slides pretreated with 0.01% polylysin (Sigma). After deparaffinization the sections were put into 0.2 M HCl (Avogadro) for 20 min, after which they were washed twice in 2×SSC, treated with 0.01% Triton X-100 (ICN) in phosphate saline buffer (PCB, ICN), washed in 0.05% Tris-HCl (pH 7.4, Sigma), and treated with proteinase K (5 μg/ml, Sigma) in 0.05 M Tris-HCl (pH 7.4) for 30 min at 37°C using Omnislide device (Hybaid).

At this stage the control section was incubated with RNase A (100 μg/ml, 0.5 M NaCl, Sigma, 10 mM Tris-HCl, pH 8.0) for 30 min at 37°C, after which washed twice in PSB.

The sections were washed for 1 min in PSB, 0.2% glycine (Sigma) was added, incubated for 1 min, washed in PSB, fixed with 0.4% paraformaldehyde (ICN) in PSB, washed in PSB, and incubated with 10 mM triethanolamine (Avogadro) and 25 mM acetanhydride (Sigma) for 10 min. After washing in PSB and water, endogenous alkaline phosphatases were blocked with 20% acetic acid (ICN) for 45 sec, after which the sections were washed in water, dehydrated in ethanol, and dried.

Prehybridization solution (10% dextrane sulfate, Sigma), 0.6 M NaCl, Denhardt solution (Sigma), 200 μg/ml salmon seminal DNA (GibcoBRL), 0.01 M Tris-HCl, pH 7.4, 0.5 mM EDTA (Serva), 50% deionized formamide were applied onto sections and incubated for 1 h at 37°C using Omnislide. Control section 2 (without probe) was covered with a slide before incubation and hermetically sealed. Prehybridization solution was removed. Hybridization solution was prepared by adding antisense probe in a concentration of 0.5 μg/ml to prehybridization solution, warming at 60°C for 10 min, and cooling in ice bath, after which it was layered onto sections. Hybridization solution with the sense probe was layered onto section 3. The sections were covered with slides and sealed. Hybridization was carried out for 16 h at 42°C using Omnislide device.

The slides were removed by plunging the samples into 4×SSC solution. The sections were incubated in 2×SSC at 42°C for 20 min and in 0.1×SSC at 45°C for 20 min using Washmodul (Hybaid), dehydrated in ethanol, dried, incubated in DIG buffer (100 mM Tris-HCl, pH 7.5, and 150 mM NaCl) for 3 min and then in 0.5% blocking reagent (Beringer) in DIG buffer for 30 min. The blocking reagent was removed, antibodies to DIG conjugated with alkaline phosphatase in 0.5% blocking reagent were added and incubated for 2 h, after which the sections were washed in DIG buffer and buffer for detection (100 mM Tris-HCl, pH 9.5, and 100 mM NaCl). The sections were incubated in NBT/BCIP solution (Boehringer), washed in water, mounted for microscopy, and the results were analyzed under an Axiovert-100 microscope (Zeiss).

RESULTS

We previously found that the production of IL-8 mRNA increased 90.2 times and that of TGF-β_2 increased 10.3 times compared to normal [1] in the FCFT of patients with PIAD. The expression of IL-10 gene in the endometrium at the medium proliferative stage also increased in comparison with the normal level (15.6 times, p=0.007).

In situ hybridization confirmed these results and showed some regularities in local production of these cytokines.

Normally, low expression of TGF-β_2 was observed mainly in epithelial cells of the endosalpinx (Fig. 1, *a*) and in some stromal cells. The level of TGF-β_2 expression in inflamed tissues notably increased not only in the epithelium, but was observed also in inflammatory infiltration macrophages (Fig. 1, *b*). The present results are in line with previous data [15] indicating that both epithelial and other FCFT cells express TGF-β group cytokines throughout the menstrual cycle. The level of expression was minimum during the early proliferation and late secretion phases. It is known also that group TGF-β cytokines play a role in tissue repair during inflammation or other tissue damage [5] and exhibit immunosuppressive effects [6]. The results of this study indicating an increase of TGF-β_2 mRNA production by epithelial cells and macrophages can be regarded as an evidence of activation

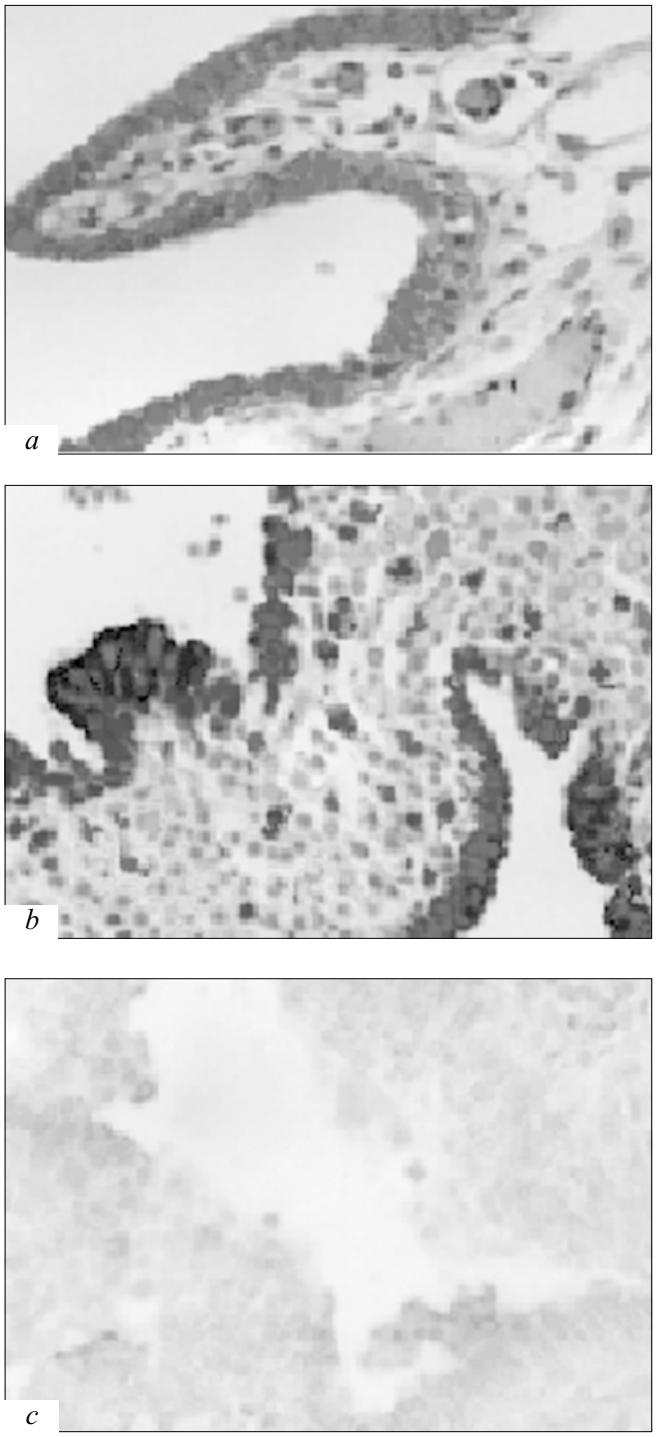

Fig. 1. Local expression of TGF-β_2 gene in the fimbrial compartment of fallopian tube in pyoinflammatory disease of fallopian tubes (PIAD), ×200. *a*) normally the gene is expressed at a low level by epithelial cells of fimbrial compartment of fallopian tubes (FCFT); *b*) during inflammation gene expression markedly increased in epithelial cells and macrophages; *c*) negative control (sense probe).

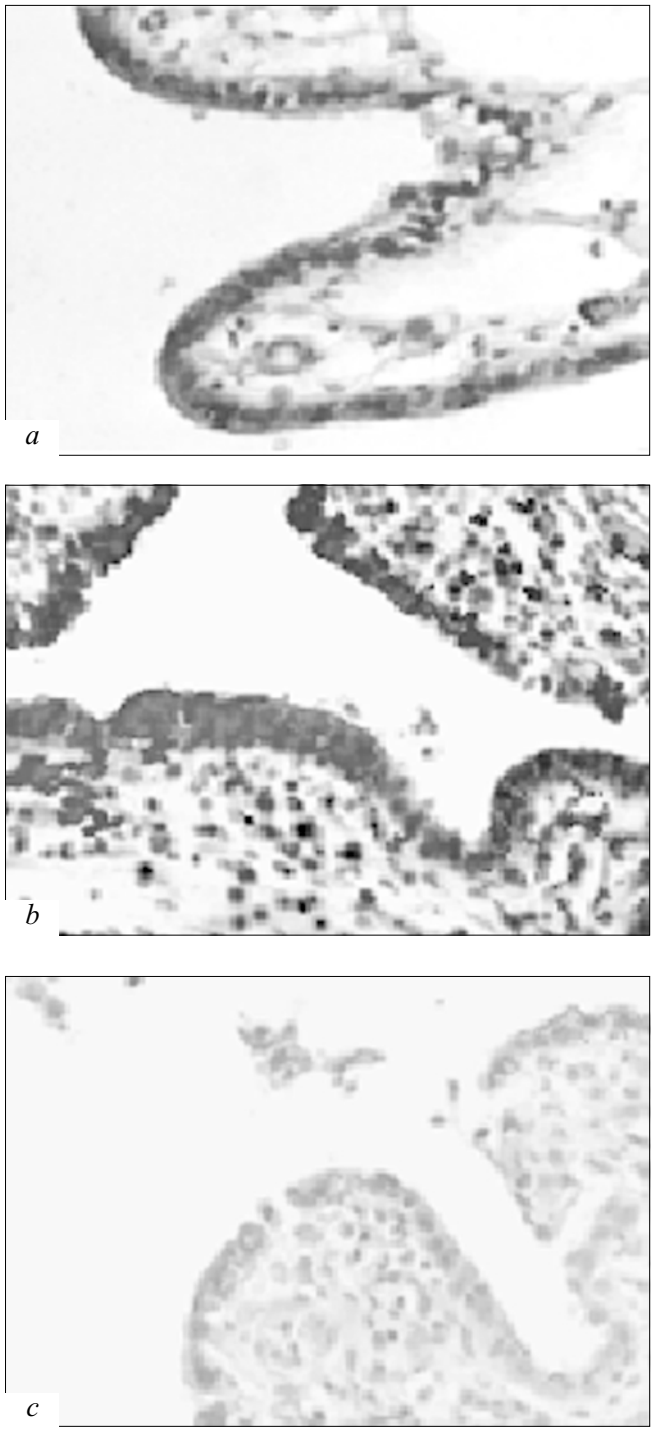

Fig. 2. Local expression of IL-8 gene in the fimbrial compartment of fallopian tube in PIAD, ×200. *a*) normally the gene is expressed at a low level by FCFT epithelial cells; *b*) during inflammation gene expression markedly increased in epithelial cells; the most active producers of IL-8 mRNA are inflammatory infiltration cells; *c*) negative control (sense probe).

of reparative and antiinflammatory processes in the fimbrial compartment.

Neutrophil migration factor IL-8, in turn, plays an important role in positive regulation of proinflammatory reaction. The expression of IL-8, which we evaluated as weak or moderate, was normally observed only in surface epithelial cells of FCFT (Fig. 2, *a*), which does not contradict published data on the

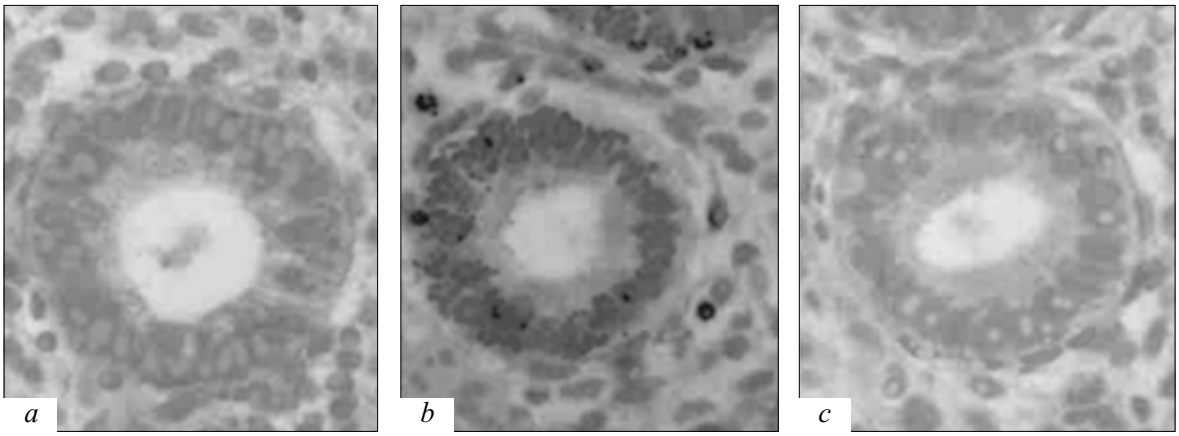

Fig. 3. Local expression of IL-10 gene in the endometrium in PIAD, ×500. *a*) normally the gene is expressed at a low level by the glandular epithelium cells; *b*) during inflammation gene expression increased in epithelial cells and few macrophages and lymphocytes; *c*) negative control (sense probe).

production of this cytokine in intact uterine tubes [11]. The development of PIAD led to a sharp increase in mRNA production for this chemokine in both epithelial cells and, most markedly, in various cells of the inflammatory infiltration (lymphocytes, neutrophils, and macrophages) (Fig. 2, *b*).

The expression of IL-10 gene was characteristic of normal endometrium (mainly of the glandular epithelial cells, Fig. 3, *a*); however it markedly increased in the endometrium with signs of inflammation in women with PIAD (Fig. 3, *b*). The content of IL-10 mRNA increased primarily in the endometrial glandular epithelial cells and solitary macrophages and lymphocytes infiltrating the stroma.

Hence, competitive activation and suppression of inflammatory process were observed in the FCFT, while in the endometrium the immune processes were aimed at prevention of inflammation and maintenance of conditions for potential implantation of a fertilized ovicell, which was seen from increased level of local expression of IL-10 gene.

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