Secretion of Proinflammatory Cytokines by Villous Chorion Tissue in Spontaneous Abortion

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Secretion of proinflammatory cytokines IL-1β, TNF-α, IL-6, IFN-α, and IFN-γ by the chorion tissue (8-12 weeks) was studied in normal gestation and spontaneous abortion. The production of IL-1β, TNF-α, and IFN-α virtually did not change in spontaneous abortion, while IFN-γ was not secreted in all experimental groups. The production of IL-6 increased more than 2-fold in patients with spontaneous abortion during the first trimester. These data confirm the involvement of this cytokine in the reproductive processes and in the pathogenesis of miscarriage.

Key Words: *cytokines; spontaneous abortion; villous chorion; tissue culture; interleukin-6*

The involvement of cytokines in the reproductive processes is no longer doubted. Cytokines of the fetoplacental complex are involved in the regulation of virtually all stages of gestation from implantation to delivery [4,8,9]. The "cytokine balance" in the uterus during normal gestation is maintained due to intricate fine interrelationships between fetoplacental complex cells and their products. Imbalance in this system induced by exogenous or endogenous factors can lead to pregnancy complications and even to spontaneous abortion [2,3]. It is known that the biological effect of this or that cytokine on target cells is determined by its concentration, and different concentrations of the same cytokine can cause opposite effects [12].

Virtually all known cytokines are present in tissues and fluids of the intrauterine compartment, but their role in the reproductive processes is not quite clear [9]. We should like to draw attention to proinflammatory cytokines IL-1, TNF-α, IL-6, IL-8, and IFN-γ, which are most often discussed in reports in association with full-term and preterm delivery. It was shown that labor is associated with appreciable changes in the concentrations of these products in the amniotic fluid and in tissue cultures of the fetoplacental complex [6,11].

The production of these molecules at the early stages of gestation in normal pregnancy and spontaneous abortion is less studied. It is extremely difficult to evaluate the contribution of certain cells and tissues into the production of cytokines *in vivo*, while the culturing approach helps to evaluate changes in the secretory activity of individual structures in different outcomes of pregnancy.

We investigates secretion of IL-1β, TNF-α, IL-6, IFN-α, and IFN-γ by chorion tissue in normal gestation and spontaneous abortion during the first trimester.

MATERIALS AND METHODS

Chorion tissue was collected after pregnancy interruption at weeks 8-12 of gestation from women aged 16-37 years. Pregnancy was spontaneous in all women. Three experimental groups were formed: normal pregnancy (group 1, n=9), spontaneous abortions (group 2, n=6), pregnancy complicated by threatened abortion (group 3, n=5).

Explants of villous chorion tissue were cultured *in vitro* in DMEM/F-12 (1:1) with 10% fetal calf serum for 24 h (37°C, 5% CO_2). After incubation cyto-

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kine concentrations in the culture medium were measured by enzyme immunoassay using Protein Contour test systems. The results are presented as the mean concentrations of secreted cytokine per mg tissue. The results were statistically processed using Student's *t* test.

RESULTS

IFN-γ was not secreted by villous chorion tissue in detectable amounts in all studied groups.

The level of IFN-γ production in cultures was not high, and in some cases the content of this cytokine in culture medium was below the detectable level. No differences in the production of this cytokine in different groups were detected.

Group 1 was characterized by appreciable heterogeneity by the levels of IL-1β secretion. The mean level of IL-1β secretion in these women was higher than in other groups (Fig. 1, *a*), but this difference was not confirmed by statistical analysis because of great scatter of experimental values.

The secretion of TNF-α by the explants (Fig. 1, *b*) was comparable with that of IL-1β (≤2 pg/mg tissue). The production of this cytokine in different groups was similar.

In contrast to other cytokines, the secretion of IL-6 in group 1 was high (mean 334 pg/mg) and in groups 2 and 3 this parameter increased significantly (2.5- and 2-fold, respectively, $p<0.05$; Fig. 1, *c*).

There is ample evidence on changes in the production of proinflammatory cytokines, primarily IL-1β, TNF-α, IL-6, IFN-γ, associated with preterm and full-term delivery. We evaluated secretory activity of villous chorion at the early stage of gestation (end of the first trimester), *i.e.* when structural organization of the placenta is completed. Presumably, the criticality of this stage determines low or zero levels of IL-1β, TNF-α, IFN-γ producing damaging effects on the trophoblast and placental vessels and known as abortion inductors [4].

The absence of IFN-γ mRNA, low expression of IL-1β and TNF-α mRNA and high level of IL-6 mRNA reported previously [7] are in good agreement with our data, except one point: low or zero IFN-α in our samples.

Similarly to preterm delivery at later terms of gestation increased secretion of the studied cytokines

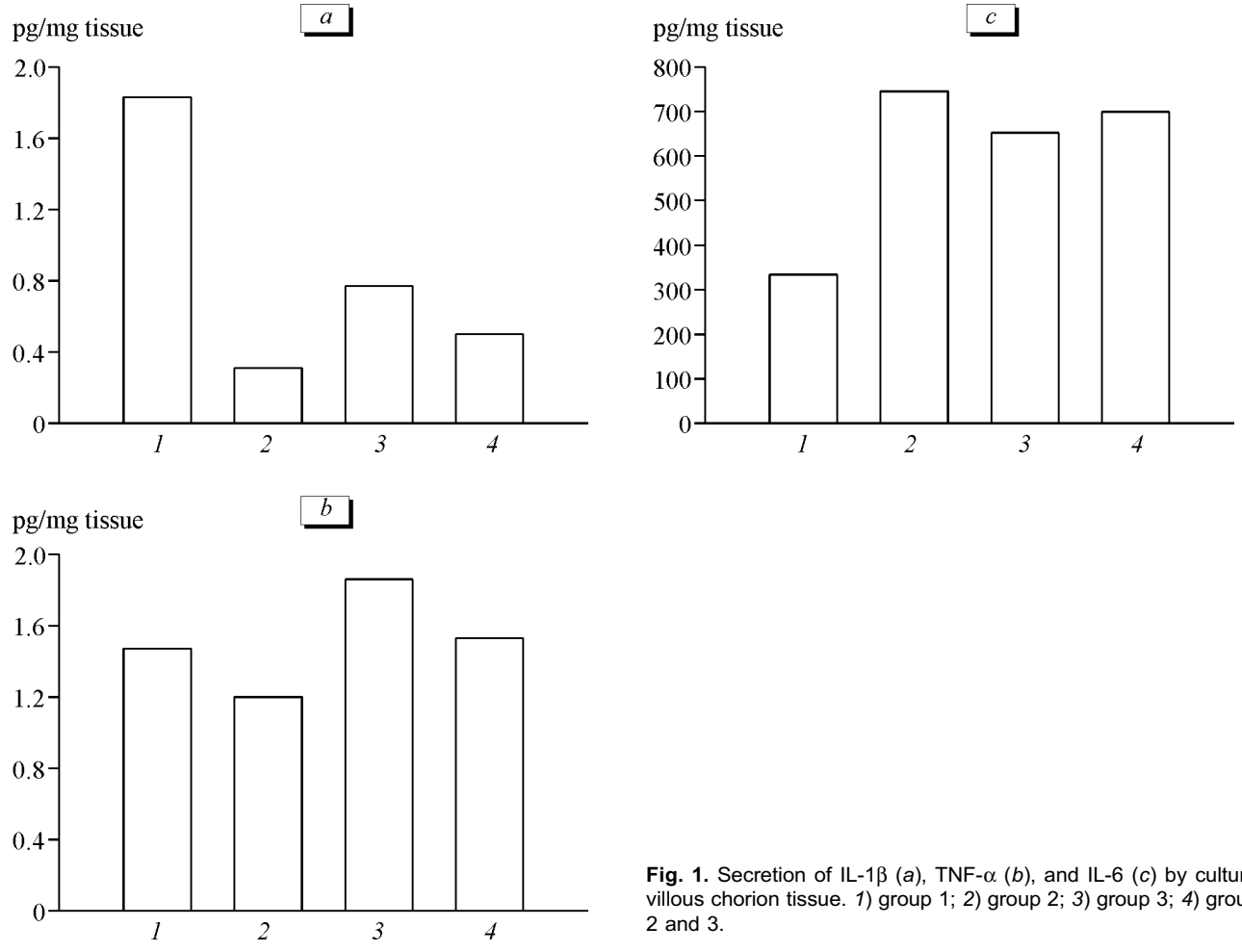

Fig. 1. Secretion of IL-1β (*a*), TNF-α (*b*), and IL-6 (*c*) by cultured villous chorion tissue. *1*) group 1; *2*) group 2; *3*) group 3; *4*) groups 2 and 3.

in early spontaneous abortion can be expected. However, only production of IL-6 significantly increased. Presumably, the spectrum of cytokines associated with abortion is different at different stages of gestation. A similar picture was observed in experiments with cultured placental macrophages: production of some proinflammatory cytokines notably increased in association with preterm delivery during the second trimester, but did not change or even decreased in full-term delivery [1].

It is known that IL-6 is constitutively produced by fetoplacental tissues almost throughout pregnancy [4]. Increased production of IL-6 by placental villi explants at later terms of gestation was demonstrated, which was associated with increased content of mRNA for this cytokine [5]. Increased production of IL-6 by choriodecidual explants in full-term delivery was observed *in vitro*, while the production of this cytokine by villous chorion tissue in labor remained unchanged [11].

We failed to identify cells maintaining the background level of IL-6 production and responsible for its increase in miscarriage. It was hypothesized that villous epithelial cells can play a role in these processes [14]. However other villous cells can also produce and release IL-6, for example trophoblast cells [10, 13] and placental macrophages [18].

An important question of a study like this is whether the observed changes in the level of this or that cytokine precede or result from abortion. The fact that IL-5 level increased not only after spontaneous abortion, but also in women with threatened abortion supports the first hypothesis.

The reproductive role of IL-6 intensively produced in the intrauterine compartment remains unclear. No doubt, IL-6 plays an important role in gestation, and its increased secretion associated with spontaneous abortion can attest to imbalance in the placental cytokine network induced by some exo- or endogenous factors, such as infection, local hypoxia, hormones, and other cytokines.

Our results confirm the relationship between spontaneous abortion and increased intrauterine level of IL-6 at different terms of gestation and confirm the hypothesis on the important role of this cytokine in the etiology of miscarriage.

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