Interleukin-17 Increased Progesterone Secretion by JEG-3 Human Choriocarcinoma Cells

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Introduction
Cytokines play a complex role at the maternal–fetal interface, involving trophoblast hormone production as well as implantation and placentation processes¹–⁵. Previous studies have demonstrated the expression of interleukin (IL)-17 in human placenta and the role of IL-17 in the invasion of JEG-3 human choriocarcinoma cell line, which expresses IL-17 receptor⁶,⁷. Nevertheless, the involvement of this cytokine in the regulation of human pregnancy remains largely unknown.

The JEG-3 choriocarcinoma cells are derived from human choriocarcinomas and retain several characteristics of normal pregnancy-derived trophoblast cells⁸. Choriocarcinoma cell lines produced various cytokines and express a range of cytokine receptors⁹–¹¹ that may involve their function such as the production of progesterone and human chorionic gonadotropin (hCG). Although macrophage and T-cell cytokines have been studied, IL-17 and its receptor have never been investigated. Progesterone and hCG produced by placental trophoblast are important in normal pregnancy. This study was therefore aimed at investigating the effect of IL-17 on the production of hCG and progesterone by the JEG-3 cells used as an in vitro model for placental trophoblast.

Method of study
The present study investigated the in vitro effect of IL-17 on progesterone and human chorionic gonadotropin (hCG) secretion by JEG-3 cells. Both hormones were quantified using enzyme-linked immunosorbent assays.

Results
The results showed that IL-17 significantly increased progesterone secretion at 6 (P < 0.001) and 24 (P < 0.01) hr, while this cytokine had no effect on hCG secretion.

Conclusion
Interleukin-17 may regulate the function of JEG-3 cells through increased progesterone secretion.
Collection (ATCC; University Boulevard, Manassas, VA, USA; ATCC number HTB-36). The cells were maintained in RPMI 1640, (Sigma, St Louis, MO, USA) containing penicillin (50 u/mL), streptomycin (50 μg/mL), L-glutamine (2 mm), and 10% heat inactivated fetal calf serum (Sigma) (complete medium), at 37°C in 5% CO₂ humidified incubator. Confluent cultures were passaged every 3 to 4 days by trypsinization with 0.25% trypsin (Sigma) in phosphate-buffered saline (PBS; Sigma).

Treatment with IL-17
To assess the effect of IL-17 on the secretion of hCG and progesterone by JEG-3 cells, the cells were trypsinized and resuspended in complete medium. Aliquots of 2 × 10⁴ cells in 200 μL complete medium were cultured in each well of 96-well flat-bottom microtiter plates (Nunclon, Roskilde, Denmark). After an overnight incubation, the cells were examined for attachment. Then the medium was removed and fresh medium containing recombinant human (rh) IL-17 (Pierce Biotechnology, Rockford, IL, USA) was added at final concentrations of 1, 10, and 100 ng/mL, each in quadruplicate wells (this range of concentration of IL-17 is recommended because it can exert activity in most in vitro applications including induction of IL-6 and IL-8 in bronchial epithelial and venous endothelial cells). Control wells are JEG-3 cells in complete medium alone. After 3, 6, 24, 48, and 72 hr, the supernatants were harvested. The supernatants were kept at −20°C until determination of hCG and progesterone.

Quantification of Human Chorionic Gonadotropin and Progesterone
The JEG-3 cell culture supernatants were analyzed by commercial enzyme-linked immunosorbent assay (ELISA) kits for the concentrations of hCG and progesterone (BioSource International Inc., Camarillo, CA, USA) according to the manufacturer’s instructions. The absorbance was read at 450 nm using a Packard SpectraCount® Microplate Photometer (PerkinElmer Life Sciences, Downers Grove, IL, USA).

Statistical Analysis
All the assays were performed on three different occasions. Statistical differences between two groups were analyzed using two-tailed Student’s t-test and the P values of less than 0.01 were considered significant.

Results
Human Chorionic Gonadotropin Secretion
The results showed that JEG-3 cells secrete hCG, which increased from 3 to 72 hr (Fig. 1). All concentrations of IL-17 did not have any effect on hCG secretion at all time points tested as compared with untreated cells (Fig. 1).

Progesterone Secretion
JEG-3 cells produced progesterone that increased with time until 72 hr (Fig. 2). A significant increase in progesterone secretion was found at 3 (P < 0.001) and 6 (P < 0.01) hr after treatment with IL-17 (Fig. 2).

Discussion
This present study demonstrated a significant stimulatory effect of IL-17 on progesterone secretion by JEG-3 choriocarcinoma cells, while this cytokine had no effect on hCG secretion by these cells. This in vitro stimulatory effect of IL-17 on progesterone secretion found only at early time points (6–24 hr) could be because of insufficient IL-17 available after

![Fig. 1](representative human chorionic gonadotropin (hCG) secretion from JEG-3 cells in response to various concentrations of rhIL-17 at 3, 6, 24, 48, and 72 hr. Each bar represents the mean concentration of hCG quantitated by enzyme-linked immunosorbent assay. Error bars are standard deviation.)
24 hr so that there was no longer sufficient receptor occupancy. Moreover, after 48 hr, the JEG-3 cells also proliferated to nearly confluent, thus their response to IL-17 might change because of cell characteristics with higher amounts of progesterone secretion. We have previously identified the presence of IL-17 receptor in JEG-3 cells and showed that JEG-3 cells responded to IL-17, in a dose-dependent manner, by increasing their invasion into the Matrigel. Progesterone may regulate trophoblast invasion, being associated with increased HLA-G expression by human trophoblast cells. The functional regulation of HLA-G on the invasion of trophoblast in normal and abnormal human pregnancy as well as trophoblast cell line has been reported. In addition, IL-17 may directly regulate the expression of matrix metalloproteinases (MMPs). These molecules are also secreted by placental trophoblast, suggesting a possible involvement of IL-17-induced MMPs in trophoblast invasion.

Limited data on the expression of IL-17 have been described in mice and humans at early gestation, when trophoblast invasion is taking place. In mice, IL-17 may be expressed in the uterine glands and basal stroma at days 6.5–10.5 of gestation. In humans, early spontaneous miscarriage placenta also expresses IL-17 that is localized to the HLA-G+ extravilous trophoblast, the villous syncytiotrophoblast and cytotrophoblast as well as villous Hofbauer cells. This suggests that IL-17 may have some functions at the feto-placental unit of early pregnancy, although its in vivo role has not been clarified. Studies have suggested that IL-17 may have a role in angiogenesis, and placental trophoblast cells as well as maternal immune cells in the uterus express large amounts of angiogenesis factors needed for successful pregnancy.

Nevertheless, the JEG-3 cell line used as a model for human trophoblast may behave differently from normal human trophoblast cells and thus the role of IL-17 in human pregnancy remains to be investigated. Thus, studies are being carried out in our laboratory to investigate the role of IL-17 in other aspects of trophoblast function, such as MMPs expression, as well as in the proliferation and function of human trophoblast from normal and abnormal pregnancies.

Acknowledgments

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References

4 Lash GE, Otun HA, Innes BA, Bulmer JN, Searle RF, Robson SC: Inhibition of trophoblast cell invasion by TGF/1, 2, and 3 is associated with a decrease in active proteases. Biol Reprod 2005; 73:374–381.