Introduction

The human placenta is a unique endocrine organ which performs essential metabolic functions during pregnancy, including the secretion of human chorionic gonadotropin (hCG) (1). GnRH and GnRH-receptor (-R) system has been shown to regulate the secretion of hCG in the human placenta (2).

Interleukin-6 (IL-6) is a cytokine with multiple biological activities. It regulates a number of cellular proliferations and differentiations in the immune and inflammatory responses (3). Recently, IL-6 is reported to possess the stimulatory activities of multiple hormones release from rat pituitary cells (4). We, therefore, investigated whether IL-6 stimulates human placental hormone secretion such as hCG. In the present study, we examined whether IL-6 and IL-6-R system is involved in the stimulation of hCG secretion. Moreover, we investigated immunohistochemically the localization of IL-6-R in the placenta.

Materials and Methods

Reagents.

Human recombinant IL-6 (hrIL-6) with $5 \times 10^6$ U/mg protein was used. The mouse monoclonal antibodies specific for IL-6 and IL-6-R have been described elsewhere (3). All of these reagents were kind gifts from Drs. T. Hirano and T. Kishimoto (Osaka University). A GnRH agonist (leuprolide acetate) was kindly gifted by Takeda Pharmaceutical Co. (Osaka).

Preparation of trophoblasts.

Fresh placentas were obtained from legal abortion.
Villous tissue was separated from connective tissue. It was incubated with Type III collagenase at 37 C for 50 min in shaking water bath (2). Cell viability estimated by trypan blue was more than 90%. Dispersed trophoblast cells were adjusted to 2 x 10^5 cells /ml. Cells were cultured for 2 days at 37 C in a humidified atmosphere of 5% CO_2 in air.

Stimulation of trophoblasts.

After 2 days culture, the supernatant of dispersed trophoblast cells was removed. The cells were then stimulated with various concentrations of either rhIL-6 or a GnRH analogue. In some experiments the cells were pretreated with PM-1 for 30 min, and then washed to be stimulated by rhIL-6 or a GnRH analogue(3).

Hormonal assays and statistics.

HCG released into medium was measured by an enzyme immunoassay (Mochida Co., Japan) using a monoclonal antibody specific for beta-subunit of hCG (2). Statistical analysis of the results for levels of significance was performed by a Duncan test following a one way analysis of variance.

Immunohistochemistry of IL-6-R.

To determine the localization of IL-6-R, immunohistochemistry technique was applied with the avidin-biotin-peroxidase complex method (PK-4002 Vectastain Laboratories, CA)(5). Briefly, freshly-frozen placental sections were incubated with MT-18 specific for IL-6-R at 4 C overnight. After rinsing with PBS, they were further incubated with biotin-labeled horse anti-mouse IgG followed by the addition of avidin-biotin-peroxidase complex at 4 C. The sites of peroxidase activity in the section were visualized with 0.1% 3,3-diaminobezidine-tetrahydrochloride containing 0.02% hydrogen peroxidase in 0.1 M Tris buffer (pH 7.2). The slide were counterstained with Meyer's hematoxylin.

Results

Figure 1 shows that rhIL-6 stimulated hCG release from placenta to the similar level with that of a GnRH analogue. The peak response of IL-6-mediated hCG
release occurred at 90 min after the stimulation. Dose response of IL-6-mediated hCG release was then examined, as shown in Fig. 2. It was statistically significant in stimulating the placenta to release hCG by 0.01 U/ml (P< 0.05) and 0.1 U/ml of hrIL-6 (P< 0.01). Any concentrations of IL-6 examined so far induced the optimal responses of hCG release at 90 min.

PM-1 is a monoclonal antibody specific for IL-6-receptor (R) and is capable of blocking IL-6-mediated signal transduction, because it binds to the smaller molecule of IL-6-R (gp-80). Figure 3 demonstrates that rhIL-6-mediated hCG release was completely blocked by the addition of PM-1. Even 0.05 g/ml of PM-1 was capable of blocking of the hCG release completely. As shown in Fig. 4, GnRH-mediated hCG release, however, was not blocked by PM-1 significantly. The data suggested the existence of two independent pathways in hCG release; IL-6-dependent and IL-6-independent GnRH-dependent pathways.

Immunohistochemical analysis of the localization of IL-6-R in the placenta was performed using MT-18, another monoclonal antibody specific for IL-6-R. Only trophoblast layer was positively stained, but neither mesenchymal cells or endothelial cells in the placenta were stained (Fig. 5).

Discussions

Our present data demonstrated that IL-6 is capable of stimulating hCG release from human trophoblasts in the first trimester. IL-6 has been reported to release multiple hormones from rat pituitary gland cells in vitro(4). Taken together with our present study, it is now clear that IL-6 has ability to release hormones in human as well as rat endocrine systems, of which evidence implys the close relationship between endocrine and immune systems.

Our present study with PM-1 and MT-18 reveals functionally and immunohistochemically the existence of IL-6-R in trophoblast surface membranes. PM-1 has been reported to recognize gp 80, one of IL-6-R, subsequently inhibiting the association with gp 130, a larger part of IL-6-R, to block IL-6-mediated responses(3). The IL-6-mediated stimulation of hCG release and its blocking by PM-1 further implicates the possible presence of gp 130 on human trophoblasts. PM-1, however, failed to block GnRH-mediated hCG release, suggesting that GnRH activates IL-6-R-
independent, GnRH-R-dependent signal transduction pathways. Our data, consequently, show existence of the two independent regulatory pathways in hCG secretion; IL-6 and IL-6-R-mediated and GnRH and GnRH-R-mediated pathways. Since we previously showed that trophoblasts in the placenta constitutively produce and secrete IL-6 during pregnancy (5), the findings suggest the involvement of IL-6 and IL-6-R systems in the local regulatory network of hCG secretion by human trophoblasts.

Figure 1

![Figure 1](image1)

Figure 2

![Figure 2](image2)

Figure 3

![Figure 3](image3)

Figure 4

![Figure 4](image4)

Figure 5

![Figure 5](image5)
References


