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THE EFFECT OF INTERLEUKIN-1α AND TUMOR NECROSIS FACTOR α ON THE SECRETION OF HUMAN CHORIONIC GONADOTROPIN BY JAR HUMAN CHORIOCARCINOMA CELLS

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The regulation of human chorionic gonadotropin (hCG) secretion by placental trophoblasts is incompletely understood. A recent study reports that Interleukin-1ß (IL-1ß) stimulates hCG production in vitro by human, first trimester, placental trophoblasts, but not by a human choriocarcinoma cell line (1). Human decidua has been shown to produce IL-1α and ß, and Tumor Necrosis Factor α (TNFα). The precise role(s) of these proteins in pregnancy is unknown. In the present study, hCG production by human choriocarcinoma cells (JAR) was evaluated in the presence of recombinant human IL-1α (rHIL-1α) and rHTNFα. hCG production was increased by rHIL-1α in a dose-dependent manner, and heat-inactivation of this cytokine abolished the effect. Equimolar quantities of rHTNFα failed to influence hCG production or cell viability. IL-1 may be important in the regulation of hCG production by human trophoblasts, and therefore may play a physiologic role in pregnancy. Furthermore, TNF does not appear to participate in the regulation of the production of this hormone by human choriocarcinoma cells. This is the first demonstration of a divergence of activity of these two cytokines in the reproductive process.

Several biological activities have been proposed for inflammatory mediators in the process of reproduction. The involvement of cytokines in ovulation (2,3), induction of labor (4,5), and the placental injury, fetal death and abortion noted in animal models of infection (6,7), have all been described. An environment potentially rich in cytokines may exist for the peri-implantation embryo and trophoblast. This is suggested by studies demonstrating IL-1 production in vitro by human decidual cells (4,5,8), placental macrophages (9,10), and trophoblasts themselves (11). Decidual cells and trophoblasts have also been shown to produce TNF when stimulated by bacterial lipopolysaccharide (LPS) (4,5).

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Post-implantation trophoblasts, which are in anatomic proximity with decidua could, therefore, be influenced by one or more cytokines. An essential function of the early post-implantation trophoblast is the production of hCG, which acts to accomplish corpus luteum rescue, thus allowing for continuation of the pregnancy (12). We hypothesized that the cytokines IL-1 and TNF might influence trophoblast hCG secretion. To test this hypothesis, human choriocarcinoma cells (JAR), a well characterized in vitro model of the endocrine trophoblast (13), were exposed to recombinant human IL-1α (rHIL-1α) and rHTNFα, and the amount of hCG secreted into the supernatant was determined.

Materials and Methods

Cell Culture Procedure

Human choriocarcinoma cells (JAR, American Type Culture Collection # HTB 144, Rockville, MD) were cultured in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with heat-inactivated (HI) fetal bovine serum (10%) (Hazleton Biologics, Inc., Lanexa, KS), penicillin (100 units/ml), streptomycin (100 ug/ml), glutamine (2mM) (Gibco) and Hepes buffer (25mM) (Hazleton). Cells were grown to near confluence in $75cm^2$ tissue culture flasks (Corning Glass Works, Corning, NY) at 37°C in a humidified atmosphere of 95% air/5% CO_2. The cells were detached with 0.5% trypsin-0.53mM EDTA (Gibco), washed once with PBS (Gibco), suspended in complete medium, and 7.5×10^4 cells in 2 ml aliquots were placed on 24-well plastic tissue culture plates (Linbro®, Flow Laboratories, Inc., McLean VA). Cell number and viability (>90%) prior to plating were determined by trypan blue exclusion. After twenty-four hours of culture, the spent medium was aspirated and replaced with 2 ml of medium alone, or medium containing cytokines or LPS. Twenty-four hours later, supernatants were harvested sterilely and stored at -70°C until assayed. In a separate experiment, cell viability was determined at the end of the experimental period by the extent of reduction of the chromogen 3-(4,5-dimethylthiazol 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) as previously described (14).

IL-1α, TNFα and LPS

rHIL-1α ($3x10^8$ units/mg protein, D10 assay; endotoxin content:<1 EU/mg protein, Limulus amebocyte lysate assay); was provided by Dr. Peter Lomedico, Hoffman-LaRoche Inc., Nutley, NJ (15); ($2.25x10^7$ u/mg as determined by murine thymocyte assay in this laboratory (16)). The units reported in these studies are those determined by the D10 assay. rHTNFα ($3x10^8$ units/mg protein, L929 assay; endotoxin content:<71 EU/mg protein, Limulus amebocyte lysate assay) was provided by the Chiron Corporation, Emeryville, CA. Both were stored at 4°C. LPS from Escherichia coli 0127:B8 was obtained from Sigma (St. Louis, MO). Stock solutions of rHIL-1α and rHTNFα containing 2×10^6 u/ml were prepared in sterile non-pyrogenic saline (Abbott Laboratories, North Chicago, IL). Aliquots of each were heat-inactivated (HI) for thirty minutes at 100°C. Stock solutions were diluted in complete medium to yield the final experimental concentrations of 500, 250, 50 and 5 u/ml of each native protein and the equivalent to 500 u/ml of each HI cytokine. A solution of complete medium was prepared containing 10 ug/ml LPS.

hCG and Cellular Protein Measurements

The concentration of hCG in 100ul aliqouts of supernatant was determined by an automated fluorometric enzyme immunoassay which detects

between 2 and 500 mIU/ml (Stratus®, Baxter Healthcare Corporation, Miami, FL) (17). After supernatant procurement, cells were washed with PBS, solubilized in 1N NaOH for 12 hours at room temperature and neutralized with 1N HCl. The protein content of the cells was then determined by the method of Bradford (18).

Statistical Analyses

The data were analyzed by the one-way analysis of variance and the Newman-Keuls test (19). $p<0.05$ was considered statistically significant.

Results

rHIL-1α increased hCG production in a dose-dependent manner. Significant elevations above control were noted at 50, 250 and 500 u/ml, with no effect evident at 5 u/ml. Heat-inactivation resulted in a return to control values (Table 1). In contrast, rHTNF failed to demonstrate any effect on hCG production over the equivalent range of doses. 10 ug/ml of LPS also failed to influence hCG production.

Total cellular protein (ug/well) did not vary significantly among the groups (103.3 ± 2.7, control; 103.8 ± 3.4, rHIL-1α 500 u/ml; 105.3 ± 1.6,

Table 1. Effects of rHIL-1α, rHTNFα and LPS on hCG production by JAR human choriocarcinoma cells

Treatment[a]	mIU hCG/mg total protein	
	Mean ± SEM	Range
Control	195.4 ± 5.6	179.0-214.3
rHIL-1α u/ml		
5	252.9 ± 10.2[b]	214.8-272.7
50	475.1 ± 35.1[c,d]	386.4-639.3
250	525.7 ± 26.5[c,e]	467.6-613.0
500	647.9 ± 19.6[c]	591.8-713.8
HI[g]	233.3 ± 12.0[f]	205.2-289.6
rHTNFα u/ml		
5	212.7 ± 6.2	191.8-235.1
50	187.3 ± 14.5	177.2-230.5
250	193.6 ± 9.6	149.5-219.5
500	238.5 ± 11.7	195.6-282.6
HI[g]	210.2 ± 16.0	174.3-286.5
LPS ug/ml		
10	203.2 ± 17.1	163.0-250.8

[a] n=6 for all groups.
[b] $p<0.01$ versus IL-1 50, 250 and 500 u/ml.
[c] $p<0.01$ versus control.
[d] $p<0.05$ versus IL-1 250 u/ml, $p<0.01$ versus IL-1 500 u/ml.
[e] $p<0.01$ versus IL-1 500 u/ml.
[f] $p<0.01$ versus IL-1 500 u/ml, $p>0.05$ versus control.
[g] equivalent to 500 u/ml exposed to 100°C for 30 minutes.

rHTNFα 500 u/ml; 101 ± 3.1, LPS 10 ug/ml). In a separate experiment, cell viability, as assessed by MTT reduction, was not affected by any reagent (IL-1, TNF 100 u/ml, LPS 20 ug/ml; data not shown). Control medium, and medium with cytokines or LPS, contained less than 0.8 mIU hCG/ml (below the limits of detection for the assay) (17).

Discussion

The data presented in this report demonstrate that the in vitro secretion of intact hCG by JAR human choriocarcinoma cells is enhanced in a dose dependent manner by rHIL-1α, but is not affected by equimolar doses of rHTNFα. The abrogation of this activity by heat-inactivation of rHIL-1α, not only demonstrates that the activity resides with the cytokine itself, but also that trace LPS contamination cannot account for the findings.

Human choriocarcinoma cell lines have maintained many of the features of normal human trophoblasts including their ability to produce hCG, progesterone and placental lactogen (13,20). Yagel et al were unable to demonstrate any effect of rHIL-1ß on the production of hCG by the same JAR cells as were used in the present study, although rHIL-1ß caused increased secretion of hCG by human placental trophoblasts (1). Several possibilities exist which could account for the differences between their findings and those of the present study. Among these are small differences in the culture media employed, and slightly different initial concentrations of JAR cells plated. Although different species of IL-1 were employed in the two studies, IL-1 receptors appear to recognize both the α and ß forms equally, and the postreceptor biological activities of the two are similar (21). Hence, the most likely explanation for the observed difference in hCG response appears to be related to dose. The maximum dose of rHIL-1ß used by Yagel et al was equivalent to the lowest dose used in the present study (comparing specific activities determined by the murine thymocyte proliferation assay). The range of doses of rHIL-1α used in the current study are similar to those exhibiting in vitro activity in other model systems (21).

The lack of activity of rHTNFα in this system is of considerable interest since IL-1 and TNF share a number of biologic activities (22). Although the anatomic opportunity for TNFα to interact with trophoblasts exists, our data suggest that this cytokine exerts no independent effect on hCG production at the concentrations examined in this study. Since the range of doses of TNF employed in the present study are equivalent to those which exhibit in vitro activity in other systems (23,24), these data suggest an important divergence for these two mediators in this situation.

This study together with the experiments of Yagel et al (1), establish that IL-1 can augment the secretion of hCG by both choriocarcinoma cells and human trophoblasts. In addition, hCG is now the third of a group of related glycoprotein hormones whose in vitro secretion is known to be influenced by IL-1. rHIL-1ß was previously shown to stimulate the secretion of luteinizing hormone and thyroid-stimulating hormone by rat pituitary cells (25).

In conclusion, it appears that IL-1 may play a role in the regulation of peri-implantation hCG secretion and thus, impact on the success or failure of early pregnancy by the maintenance of an appropriate hormonal background (12,20). The potential interactions between IL-1 and other recognized hCG secretagogues, such as epidermal growth factor (20) and fibroblast growth factor (26), remains to be determined. The effects of other cytokines (e.g. interleukin-6) alone, or in combination with IL-1, will require further investigation. In addition, it is possible that cytokines produced locally by the decidua may participate in the regulation of the production of other trophoblast secretory products, such as progesterone and placental lactogens.

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