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Human Chorionic Gonadotropin Contributes to Maternal Immunotolerance and Endometrial Apoptosis by Regulating Fas-Fas Ligand System

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The first known hormonal signal of the conceptus during implantation is human chorionic gonadotropin (hCG). Interestingly, increased apoptosis in human endometrium coincides with the implantation window. Factors from the fetal or placental origin as well as maternal hormonal factors are likely to have a potential role in the regulation of apoptotic signaling molecules. We hypothesized that hCG may be a placential factor for the development of local maternal immunotolerance. Fas-Fas ligand (FasL) system is one of the apoptotic signaling pathways, shown to be important in the development of local immune tolerance during and after implantation. We report that hCG treatment decreases cell proliferation and increases apoptosis in endometrial cells. Moreover, hCG stimulates FasL mRNA and protein expression without affecting Fas mRNA in these cells. Interestingly, in coculture experiments, hCG-treated endometrial cells induce an increase in T cell apoptosis. Our in vivo results reveal that cells of early pregnancy decidua express strong FasL immunoreactivity, and decidual areas containing interstitial cytotrophoblasts have numerous TUNEL-positive cells. Compared with decidual areas devoid of interstitial cytotrophoblasts, we observed in decidual areas containing interstitial cytotrophoblasts clearly less amount of TUNEL-positive cells. These results suggest that hCG may be a link in the development of peritrophoblastic immune tolerance and may facilitate the trophoblast invasion by regulating proapoptotic molecules such as FasL in endometrial cells. The Journal of Immunology, 2003, 171: 2305–2313.

The embryo and trophoblast unit, with distinctive immunologic and genetic characteristics, is a semiallograft to the maternal immune system, but is not counteracted with the maternal immune rejection response. Differentiation and apoptosis of endometrial cells during the decidualization of the receptive endometrium are suggested to be crucial for the immune tolerance to the pregnancy and for the limited invasion of trophoblasts (1).

During pregnancy, considerable amount of macrophages and lymphocytes is incurred around the maternal-fetal interface. Large granular lymphocytes, T lymphocytes, and macrophages comprise 70, 20, and 10% of leukocyte population, respectively, in the early decidua (2). These cells, in direct contact with invading trophoblasts, are believed to be suppressed, resulting in the development of immune tolerance at the maternal-placent interface. Fas/Fas ligand (FasL)3 signaling in activated T lymphocytes induces an immune-privileged environment in Sertoli cells (3) and the cornea (4). Fas-FasL interaction has been recently suggested as a similar mechanism for the immune tolerance at the decidua-trophoblast interface (5, 6). At both autocrine and paracrine manner, binding of FasL to its receptor Fas is possible to induce apoptotic signaling in Fas-expressing cells.

Maternal steroid hormones have a critical role in the establishment of receptive endometrium. Selam et al. (7) have shown a cycle-dependent regulation of FasL expression in human endometrium, and demonstrated that FasL is up-regulated by estrogen and progesterone in endometrial stromal and glandular cells. Previous studies on this subject revealed a potential role of the embryo as well as in the epithelial cell apoptosis during the trophoblast invasion in human (8). Apoptosis of glandular cells peaks just at the time of implantation window, ~19–20th days of the menstrual cycle, suggesting a role for glandular apoptosis in human implantation (9). The first known hormonal signal of the conceptus is human chorionic gonadotropin (hCG) detected on days 7–9 after luteinizing hormone (LH) surge, which corresponds to days 19–21 of the menstrual cycle. Human endometrium contains membrane-bound hCG/LH receptors (10), and hCG has direct actions on the decidualization of human endometrial stromal cells (11).

During rodent blastocyst implantation, cellular differentiation and subsequent degeneration of the decidua spread from the antimesometrial region to the mesometrial region to accommodate the developing conceptus (1). Moreover, in pseudopregnant mice, decidual reaction develops and also undergoes regression similar to that observed in normal pregnancy, suggesting that during decidual apoptosis paracrine factors derived from the uterus and/or from exogenously given factors, such as pregnant mare serum gonadotropin, are as important as other trophoblast-secreted factors (12, 13). We hypothesized that hCG may be involved in maternal immunotolerance and decidual apoptosis by regulating Fas and FasL expression in human endometrium. We have shown that hCG-treated endometrial cells induced apoptosis in both endometrial and T cells (Jurkat) in autocrine and paracrine manner, respectively, suggesting that hCG may take part in the development of peritrophoblastic immune tolerance.

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3 Abbreviations used in this paper: FasL, Fas ligand; CG, chorionic gonadotropin; DAPI, 4′-6-diamidino-2-phenylindole; hCG, human CG; LH, luteinizing hormone.

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Materials and Methods

Tissue collection

Endometrial tissues were obtained from human uteri after hysterectomy conducted for benign diseases other than endometrial disease or from endometrial biopsies. Human decidua tissues from first trimester (n = 6) were collected from clinically normal pregnancies, which were voluntarily terminated by dilation and curettage. Informed consent in writing was obtained from each patient before surgery; consent forms and protocols were approved by the Human Investigation Committee of Yale University. Mean age of the patients was 44.6 (range 32–50). Diagnoses of patients were leiomyomata or voluntary sterilization by tubal ligation. Each experimental age of the patients was 44.6 (range 32–50).

Mean concentration from each patient before surgery; consent forms and protocols were collected from clinically normal pregnancies, which were voluntarily initiated.

Isolation and culture of human endometrial stromal and glandular cells

Endometrial stromal and glandular cells were separated and maintained in monolayer culture, as described previously (14). Stromal cells were plated in DMEM (Sigma-Aldrich, St. Louis, MO) and FBS (10% v/v; Life Technologies, Rockville, MD).

Endometrial stromal cells after first passage were characterized, as described previously (15), and were found to contain 0–7% epithelial cells, no endothelial cells, and 0.2% macrophages. Endometrial glandular cells (largely intact glands and sheets of surface epithelium) were collected by backwashing the sieve, plated in six-well plates, previously coated with growth-factor-reduced Matrigel (Sigma-Aldrich). Cells were maintained in DMEM containing l-valine (16) (substituted for L-valine to inhibit stromal cell growth). Experiments with stromal and glandular cells were conducted 1–3 days after confluence was attained. Cells were treated with serum-free, phenol red-free medium for 24 h before treatment with test agents was initiated.

Cells were treated with various concentrations of hCG (100, 200, 500, 1000 mIU/ml; Sigma-Aldrich) for 3–48 h. Each experiment was repeated on four occasions, with similar results using endometrial cells obtained from different patients. Representative figures are presented for each experiment.

Immunohistochemistry and immunocytochemistry

Tissues for immunohistochemistry were fixed in 4% paraformaldehyde for 6 h. Paraffin blocks were cut at 5–7 μm thicknesses and mounted on gelatin-coated slides. Thereafter, sections were deparaffinized and rehydrated in alcohol gradient. For the detection of FasL (two different Abs were purchased from Transduction Laboratories and Novacastra Laboratories, New Castle, U.K.), immunohistochemistry and immunocytochemistry were conducted using a standard streptavidin-biotin technique. The immunoreaction was developed using 3-amin-9-ethylcarbazole chromogen (Vector Laboratories, Burlingame, CA), as described previously (7). Mouse IgG and mouse IgM Ab isotypes were used for the negative isotype control. Cells cultured in chamber slides were fixed in 4% paraformaldehyde without permeabilizing for immunofluorescence staining. Then cells were washed with PBS twice. Following incubation with 10% normal goat serum, immunofluorescence staining was performed by using mouse anti-Fasl as primary Ab and anti-mouse FITC-labeled Ab as secondary Ab and 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining.

The evaluations were recorded as percentages of positively stained cells in each of four intensity categories, which were denoted as 0 (no staining), 1′ (weak, but detectable above control), 2′ (distinct), 3′ (intense). For each slide, an NCORE (cell score) value was derived by summing the percentages of cells that stained at each intensity multiplied by the weighted intensity of the staining (NCORE = Σ (i + 1), where i is the intensity score and Pi is the corresponding percentage of the cells). Two observers blinded to treatment data performed the NCORE, and the interindividual variation was 16% for Fasl staining.

FasL Western blot analysis

Total protein from cells was extracted in a lysis buffer (Pierce, Rockford, IL). The protein concentration was determined by a detergent-compatible protein assay (Bio-Rad Laboratories, Hercules, CA). The electrophoresis and immunoblot were performed, as described previously (17). The signal was developed using tetramethylbenzidine peroxidase substrate kit (Vector Laboratories) and chemiluminescent kit (NEN Life Science, Boston, MA). The FasL signal was normalized by dividing the arbitrary densitometry units for Fasl to the amount of GAPDH signal (GAPDH Ab was purchased from Santa Cruz Biotechnology, Santa Cruz, CA) for each band. The Fasl and GAPDH signals were quantitated by a digital imaging and analysis system (Alpha Ease; Alpha Innotech, San Leandro, CA) for gel bands, and by using a laser densitometer (Molecular Dynamics, Sunnyvale, CA) for the autoradiographic bands.

Fas and Fasl RT-PCR

Total RNA was extracted by TRIzol reagent (Life Technologies), according to manufacturer’s instructions. A total of 2 μg of sample was reverse transcribed in 20 μl of reaction mixture containing 10 mM each of dATP, dCTP, dGTP, and dTTP; 20 pmol oligo(dT); 40 U/μl RNase inhibitor; and 10 U/μl avian myeloblastosis virus reverse transcriptase and buffer (42°C, 60 min; 95°C, 5 min; PerkinElmer model 9600 thermocycler, Norwalk, CT). RT-PCR was conducted, as described previously (15).

FasL primers yielding 311-bp reaction product: sense, 5′-ACA CCT ATG GAA TTG TCC TTC TGG-3′ and antisense, 5′-GAG CAC AGA GAG CTC AGA TAC G-3′. FasL primers yielding 266-bp reaction products: sense, 5′-CAC TAT TGC TGG AGT CAT G-3′ and antisense, 5′-CTG AGT CAC TAG TAA TGT CC-3′. F3PDH primers yielding 788-bp product: sense, 5′-GGT CGG AGT CAA CGG ATT TTG-3′ and anti-sense, 5′-CTT CCG ACG CCT GCT TCA CCA-3′.

Cell proliferation assay

Cell proliferation was also determined in 96-well plates by a colorimetric assay using MTT (Sigma-Aldrich). The first column of each 96-well plate did not contain any cells and was used as a blank. Consecutive columns were treated with hCG (200, 500, 1000 mIU/ml) and medium alone as the control group. Cell proliferation was evaluated following 24 and 48 h of treatment. MTT assay was conducted, as described previously (18). Data were expressed in OD units. Experiments were conducted with replicates of eight wells per treatment condition. Similar experiments were conducted on at least three different occasions with cells prepared from three different tissues obtained from three different patients.

DAPI staining

Endometrial stromal cells were treated with hCG (200 or 1000 mIU/ml) or serum-free medium alone as the control group for 24 and 48 h. Thereafter, cells were washed with PBS, fixed with 70% ethanol for 20 min at room temperature, and washed again with PBS. Cells were then treated with DAPI (1 μg/ml; Sigma-Aldrich) for 10 min, washed with PBS for 10 min, and mounted with mounting medium (Vector Laboratories).

Co-culture of endometrial stromal cells and Jurkat cells

Following 24-h treatment of cells with and without hCG, the medium was removed, plates were washed with PBS, and nonadherent human immortalized T lymphocyte cells (Jurkat cells; 2 × 10⁶ cell/ml) were plated either alone or on endometrial stromal cells. After 16 h of incubation, Jurkat cells in the medium were collected and fixed in 4% paraformaldehyde for 10 min. Subsequently, cells were labeled with TUNEL, as described below. Experiments were repeated on three occasions using different endometrial tissue samples each time.

TUNEL in situ apoptosis detection assay

Apoptosis in decidual tissues, endometrial stromal cells, and Jurkat cells was detected by enzymatic labeling of DNA strand breaks using TUNEL. TUNEL was conducted, as described previously (15). Jurkat cells were fixed in 4% paraformaldehyde for 5 min in microcentrifuge tubes, and all TUNEL procedures were conducted in these tubes. Alkaline phosphatase-labeled TUNEL probe reactivity was developed using Fast Red in a microwave. TUNEL labeling was conducted using a Cell Death Detection kit (Roche; Mannheim, Germany) and performed according to the manufacturer’s instructions. Quantification of apoptotic cells was accomplished either by counting or colorimetrically using microparticle reader.

Statistical analysis

Levels of FasL immunocytochemistry scores, Western blot, and RT-PCR densitometries, TUNEL assay, and MTT proliferation assay data were normally distributed, as tested by Kolmogorov-Smirnov test. Thus, ANOVA and post hoc Tukey test for pairwise comparisons were used for statistical analysis. Value of p < 0.05 was considered to be significant. Statistical calculations were performed using Sigma stat for Windows, version 2.0 (Jandel Scientific, San Rafael, CA).

Results

Regulation of FasL protein expression by hCG

Endometrial stromal and glandular cells plated onto chamber slides were incubated with hCG for 24 h and were analyzed by

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immunocytochemistry. FasL immunoreactivity was observed as membranous and perinuclear in stromal and glandular cells (Fig. 1). In untreated endometrial stromal cells, FasL immunoreactivity was weak and mostly localized in perinuclear areas (Fig. 1a). hCG (1000 mIU/ml) induced an increase in both intensity and membranous expression of FasL immunoreactivity (p < 0.05) (Fig. 1b). FasL expression was also observed weakly in untreated endometrial glandular cells (Fig. 1c). The intensity and the number of positive cells for FasL immunoreactivity were also increased by hCG (500 mIU/ml) in these cells (p < 0.05) (Fig. 1d).

To investigate the regulation of FasL protein expression by hCG, stromal cells were incubated with various concentrations of hCG (200, 500, 1000 mIU/ml) for 24 h, and FasL protein levels were analyzed by Western analysis. Untreated endometrial stromal cells expressed FasL protein (Fig. 2a). hCG treatments, at 200, 500, 1000 mIU/ml concentrations, stimulated the FasL expression to levels 34, 48, and 55% above control cell levels, respectively (Fig. 2a; p < 0.05 between control and treatments).

In separate experiments, endometrial glandular cells were incubated with hCG (200, 500 mIU/ml), FasL expression was weak in untreated endometrial glandular cells (Fig. 2b). Similar to endometrial stromal cells, hCG also up-regulated FasL expression in glandular cells (22 and 29% increase above control cell levels by 200 and 500 mIU/ml hCG, respectively; p < 0.05 between control and treatments; Fig. 2b).

**Regulation of Fas and FasL mRNA expression by hCG**

To evaluate whether the up-regulatory effect of hCG on FasL protein expression was due to an increase in FasL transcription, we measured FasL mRNA levels by RT-PCR analysis. We also investigated FasL receptor (Fas) mRNA expression in endometrial cells.

Endometrial stromal cells were incubated with various concentrations of hCG (200, 500, 1000 mIU/ml). Untreated endometrial stromal cells expressed Fas mRNA (Fig. 3a). We did not observe any alteration in Fas mRNA levels by increasing concentrations of hCG. Similarly, hCG did not demonstrate any concentration-dependent alterations in FasL mRNA expression in human endometrial stromal cells (Fig. 3b).

HCG up-regulated FasL mRNA expression in endometrial glandular cells. HCG treatments, 500 and 1000 mIU/ml induced 69 and 81% increase, respectively, in FasL mRNA levels compared with untreated endometrial glandular cells (p < 0.05) (Fig. 4a). Similar to stromal cells, we did not observe any alteration in Fas mRNA levels by increasing concentrations of hCG in glandular cells (Fig. 4b).

**Regulation of endometrial stromal cell proliferation by hCG**

Endometrial stromal cells were incubated in serum-free medium for 24 h, then were treated with various concentrations of hCG (200, 500, 1000 mIU/ml) or with vehicle (control) for 24 and 48 h, and cell proliferation was evaluated by MTT colorimetric assay. After 24 h of hCG treatment, there was a significant decrease in cell proliferation (p < 0.05 between control and hCG treatment of 1000 mIU/ml, Fig. 5). After 48 h of treatment, the significance in cell proliferation was also only at 1000 mIU/ml hCG concentration (p < 0.05, Fig. 5).

**Regulation of apoptosis in endometrial stromal and Jurkat cells by hCG**

Using DAPI staining by fluorescence microscopy, morphological indicators of apoptosis such as cell shrinkage, nuclear segmentation, and chromatin condensation were analyzed. HCG-treated (1000 mIU/ml) endometrial stromal cells had more cell shrinkage, nuclear segmentation, and chromatin condensation compared with control cells at 24 h (Fig. 6, a vs c) and 48 h (Fig. 6, d vs e). However, there was no significant difference between cells treated with hCG at 200 mIU/ml concentration and control cells (Fig. 6, a vs b). Similar to DAPI staining, in situ TUNEL labeling showed that treatment of endometrial stromal cells with hCG (1000 mIU/ml)
ml) induced an increase in TUNEL-positive endometrial stromal cell number following 48-h treatment (p < 0.01, Fig. 6).

We also investigated the indirect paracrine effect of hCG on T cells by way of inducing FasL expression in endometrial cells. Endometrial stromal cells were treated with hCG (200 and 1000 mIU/ml) for 24 h. Then nonadherent human T lymphocytes (Jurkat cells) were added over endometrial stromal cells after changing the medium. Cells were incubated together for 16 h without any hCG. Pretreatment of endometrial stromal cells with 200 and 1000 mIU/ml hCG induced 1.6- and 3.5-fold increase in the apoptotic rate, respectively; of Jurkat cells compared with Jurkat cells cultured with untreated endometrial stromal cells (p < 0.01) (Fig. 7).

**Discussion**

Regulation of apoptosis involves complex set of events by way of interaction of several genes with stimulatory and inhibitory effects on programmed cell death. Fas, also called APO-1 or CD95, is a type I membrane protein that belongs to the TNF/nerve growth factor receptor family (17). FasL, a type II membrane protein, belongs to the TNF superfamily (19). FasL expression has been...
reported in nonhemopoietic cells, mainly from immune-privileged tissues, including testis, cornea, trophoblast, and cancer cells, suggesting that the Fas-FasL system may play an important role in the mechanism underlying this immune-privileged status (3–5, 20, 21). Recently, we have described a cycle-dependent regulation of FasL expression in human endometrium, and demonstrated that estradiol and progesterone in endometrial stromal and glandular cells up-regulate FasL in both mRNA and protein level (7).

hCG, a glycoprotein hormone primarily produced by human placenta, has functional similarities with human LH, another glycoprotein hormone and a product of the anterior pituitary gland (22). Both hCG and LH bind to the same transmembrane glycoprotein receptor and stimulate steroidogenesis in gonadal cells (23, 24). hCG/LH receptors are present in glandular, luminal epithelial, and stromal cells of endometrium, and myometrial and vascular smooth muscle cells of myometrium (25). Luminal and glandular epithelial cells contain more hCG/LH receptors compared with the stromal, myometrial, and vascular smooth muscle cells. The content of hCG/LH receptors in human uterus increases in secretory phase compared with proliferative phase, suggesting a hormonal regulation. These receptors also exist in the placenta, fetal membranes, and decidua, with increased expression in syncytiotrophoblasts and decidua compared with cytotrophoblasts and fetal membranes, respectively (25). Moreover, during the first weeks of pregnancy in vivo hCG concentration is ~100–5000 mIU/ml in the plasma, which covers the hCG concentrations we have used.

Function of hCG on steroidogenesis in gonadal tissues (23, 24) and fetal adrenal gland (26) is well known. However, the existence of hCG/LH receptors in nongonadal reproductive tissues, including the endometrium and decidua, suggests a direct extragonadal effect of hCG by means of endocrine, autocrine, or paracrine mechanisms. Use of hCG for ovulation induction (11) and in premature ovarian failure promotes decidual changes in the endometrium. LH and hCG increase cAMP levels (11), activate protein kinase A, and increase PGE2 and the transcription rate of prolactin gene, thereby inducing morphological and functional differentiation of endometrial stromal cells into decidua (27). Decidual cells are also believed to produce factors that control trophoblast invasion and protect the pregnancy from maternal immune rejection. It has been previously shown that cAMP is a critical mediator of apoptosis in thymocytes and provides a molecular explanation for how the cAMP stimulators may modulate T cell production output under physiological and pharmacological conditions (28).

Up-regulation of FasL by hCG in human endometrium could have several roles in implantation. One potential role is the stimulation of glandular and stromal cell apoptosis, thus allowing the trophoblastic invasion into the endometrium. Our hypothesis is supported by the significant decrease observed in endometrial stromal cell proliferation following hCG treatment. Moreover, our observation in hCG-treated cells revealing increased morphological features of apoptosis, such as cell shrinkage, nuclear segmentation, and chromatin condensation, suggests that the decrease in cell proliferation could be due to increased apoptosis. Differentiation and apoptosis of stromal and glandular cells during decidualization of the receptive endometrium are crucial for the controlled invasion of trophoblasts and their intimate contact with maternal blood vessels (3, 29). Our results suggest that the increase in the apoptosis of glandular and stromal cells during the implantation window...
may be related to the up-regulatory effect of hCG on FasL expression besides the similar effect of ovarian sex steroids (7). Resembling steroids, vitamin D3 (1,25(OH)2 D3), a secosteroid hormone, has well-known immunosuppressive activities. Recently, it has been demonstrated that this secosteroid negatively regulates FasL expression T cells, explaining another molecular approach for hormone-mediated paracrine immunosuppressive mechanisms (30).

The distribution of LH/hCG receptors has been recently described throughout several pathologic events in the uterus. Invading trophoblasts from hydatiform mole and choriocarcinoma contain...
and overexpress hCG receptors and also secrete higher amounts of hCG compared with normal trophoblasts (31). HCG is suggested to have a role in trophoblast transformation, growth, and invasion in gestational trophoblastic neoplasias. All grades of endometrial carcinomas contain a greater abundance of LH/hCG receptors compared with secretory phase endometrium. The number of receptors is observed to be higher with increasing histological grade of tumor. LH/hCG receptor expression is speculated to be closely related to an aggressive phenotype in endometrial carcinoma (32). In adenomyosis, invading glands selectively express more LH/hCG receptor mRNA and/or immunoreactive receptor protein compared with noninvading glands in the same endometrium (33). Similarly, the ectopic endometrial implants express both LH/hCG receptor mRNA and protein in endometriosis (34). In all these pathologic events mentioned above, hCG receptor mRNA and protein expression demonstrates a correlation with an invasive characteristic of the tumor. Up-regulation of FasL expression with hCG may have a paracrine role in the invasion of these tumors as well as in the invasion of trophoblasts in early pregnancy by means of eliminating cytotoxic T lymphocytes and promoting the survival against the immune rejection response. Present results show that not only trophoblasts, but also maternal decidual and glandular

FIGURE 8. Representative immunohistochemical staining for FasL and TUNEL for in situ detection of apoptotic cells in serial sections of early pregnancy decidua. FasL immunoreactivity was mostly membranous and less cytoplasmic. Both decidual and glandular cells were positive for FasL (a, b). There is a stronger FasL immunoreactivity in apical cell membrane compared with basal pole of glandular epithelial cells (b). TUNEL-positive decidual leukocytes, stromal, and glandular cells in interstitial cytotrophoblast-positive decidual area (e), and in decidual area in which interstitial cytotrophoblasts are not observed (f). Cytokeratin-7-immunoreactive cells of decidual area with and without interstitial cytotrophoblasts are seen, respectively (c, d). Original magnification: a–f, ×100.

FIGURE 7. Representative graph of endometrial stromal and Jurkat cells coculture experiment. Endometrial stromal cells were treated with medium only (control) or with hCG for 24 h, then were cocultured with Jurkat cells. Apoptotic Jurkat cells were quantified by colorimetric TUNEL assay. Pretreatment of endometrial stromal cells with hCG (200 and 1000 mIU/ml) increased TUNEL-positive Jurkat cell number (p < 0.01).
cells may take part in the immunotolerance. Recently, Makrigianakis et al. (35) have shown that FasL expression is closely associated with a marked decrease in the number of implantation sites and live embryos. Antalarmin, a corticotropin-releasing hormone receptor type 1 antagonist, decreased both FasL expression and corticotropin-releasing hormone-induced apoptosis of activated T lymphocytes. These findings support the hCG-mediated maternal immunotolerance hypothesis.

Many studies have demonstrated the FasL expression in human trophoblasts and have suggested a role in the apoptosis of maternal T lymphocytes allowing maternal immunotolerance to pregnancy (5, 6, 20). Immunosuppression at the maternal-fetal interface was suggested to be similar to the immune privilege around Sertoli cells of the testis (3) and cornea (4). Activation of T lymphocytes by foreign Ags induces the expression of Fas, which upon binding to FasL initiates a cascade of the apoptotic pathway that eliminates lymphocytes and suppresses the immune response (36, 37). Another possibility is that the maintenance of soluble FasL on extra-cellular matrix and/or microenvironment in conjunction with the form and level of FasL expressed is likely to play an important role in the development of peripheral tolerance in immune-privileged sites (38–40). During early pregnancy, the number of uterine leukocytes increases. However, the main leukocyte types that are increased in the decidua are uterine NK cells and macrophages (41). One study comparing Fas expression and apoptosis ratio in T cells in nonpregnant and pregnant women revealed that although peripheral blood T cells expressed higher Fas during pregnancy, they became more resistant to Fas-mediated apoptosis (42). In contrast, according to our results, Jurkat cells are induced to undergo apoptosis by endometrial stromal cells in coculture. There is certainly a possibility that there also may be a resistance to Fas-mediated apoptosis in some subtypes of decidual leukocytes, as observed in peripheral leukocytes. Further studies need to be conducted to understand better the molecular basis of leukocyte-endometrial cell interactions.

Previous studies have reported both apoptotic and antiapoptotic properties of hCG. Samaniego et al. (43) have reported that hCG treatment induced apoptosis in Kaposi’s sarcoma cells both in vivo and in vitro, suggesting a role for hCG and hCG-associated factors in pregnancy-related regulation of cell death. That study showed an increase in TUNEL-positive cell number, nuclear fragmentation, and elevated expression of apoptotic factors such as c-myc, and a reduction in antiapoptotic proteins such as bcl-2 expression (43). In another study, hCG inhibited the proliferation of human breast epithelial cells by stimulating the expression of apoptotic genes, which became evident before the detection of cell growth inhibition. Moreover, gene activation differed among immortalized and chemically transformed cells, suggesting that hCG might use both p53-dependent and p53-independent pathways for inhibiting cell cycle progression (44). Our finding of the presence of apoptotic cells in decidual areas in the absence of cytrophoblasts suggests that endocrine and paracrine factors may play a role in the induction of apoptosis besides a direct trophoblast-decidual cell interaction. However, Fazleabas et al. (45, 46) have studied extensively the role of recombinant hCG in endometrial cell differentiation both in vivo and in vitro. Chorionic gonadotropin (CG) has different effects on the three cell types of endometrium (luminal, glandular epithelial, and stromal cells). The primary effect of CG on stromal fibroblasts is the induction of α-smooth muscle actin (45). It has been speculated that the induction of α-smooth muscle actin by CG may be essential to decrease the progesterone-regulated proliferation process in these cells and initiate the differentiation process, which is a prerequisite for decidualization (45). Disruption of the cytoskeleton with cytochalasin D in the absence of hormones results in a progressive increase in annexin V staining, indicating that destabilization of actin filaments results in apoptosis. Treating cells with either estradiol or progesterone can reverse this response. When actin filaments are disrupted in the presence of both steroid hormones and CG, not only are these cells rescued from undergoing apoptosis, but also their differentiation into decidual cells is enhanced (46).

In conclusion, we have demonstrated that FasL is up-regulated by hCG in endometrial stromal and glandular cells, and that hCG may induce apoptosis in T cells and endometrial cells both in an autocrine and paracrine manner. We conclude that the modulation of FasL by placental as well as maternal hormonal milieu may induce apoptosis in decidual cells, glandular cells, and immune cells, which may contribute to both the controlled trophoblastic invasion and peri-trophoblastic immunotolerance.

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