Roles of the Insulinlike Growth Factor Family in Nonpregnant Human Endometrium and at the Decidual:Trophoblast Interface

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ABSTRACT—The insulinlike growth factor (IGF) family is believed to be important in endometrial development during the menstrual cycle and in the process of implantation. The mitogenic, differentiative, and antiapoptotic properties of the IGFs and their binding proteins, as well as their spatial and temporal expression in cycling endometrium, suggest that they may participate in endometrial growth, differentiation, apoptosis, and perhaps angiogenesis. IGFBP proteases, which increase IGF bioavailability, have been localized to endometrial stromal cells and to the human cytotrophoblast and likely play important roles in endometrial, decidual, and trophoblast physiology. IGFBP-1 is a major protein product of nonpregnant endometrium during the mid-late secretory phase and occurs in abundance in decidua. Its roles as an IGF-binding protein and as a trophoblast integrin ligand suggest that it may have multiple roles in endometrial development and in interactions between the decidua and the invading trophoblast. Recent evidence suggests that it may have a role in the process of shallow implantation in the clinical disorder of preclampsia. In contrast to knowledge about the roles of IGF peptides, IGFBP proteases, and IGFBPs in normal endometrial development and early human pregnancy, little information is available regarding this family in abnormal endometrial development, in occult endometrial defects, and in uterine receptivity and nonreceptivity.

KEYWORDS: IGF, IGF binding protein, endometrium, decidua, pregnancy, implantation

THE INSULINLIKE GROWTH FACTOR FAMILY

The insulinlike growth factor (IGF) family is comprised of IGF-I and IGF-II peptides, six high-affinity IGF binding proteins (IGFBP-1 through IGFBP-6), four low-affinity IGFBPs, a family of IGFBP proteases, and at least two IGF receptors.1-3 IGF-I and IGF-II have mitogenic, differentiative, metabolic, and antiapoptotic actions and thus may serve multiple roles in cellular behavior.4 IGF-I's effects on cellular mitosis have classically been attributed to its ability to promote progression through G1 to the S-phase of the cell cycle.5,6 Recently, this...
has been challenged in studies with the igf1 null mouse. Estradiol administration resulted in an increase in cells in the S-phase in both in wild-type and mutant mouse uterus. However, there was a profound decrease in cells in mitosis in all regions of the igf1 null uterus, compared to wild-type, suggesting that IGF-I is critically involved in the progression from S-phase through the G2/M phase of the cell cycle.

The IGF receptors have affinities for the IGFs in the range of $10^{-9}$ to $10^{-10}$ M. The type I IGF receptor is homologous with the insulin receptor and is the principal receptor for signal transduction via activation of the tyrosine kinase pathway. Subsequent to insulin-receptor substrate phosphorylation, IGF mitogenic effects are primarily mediated via the MAP kinase pathway, whereas IGF metabolic effects are primarily mediated via the PI3 kinase pathway. The type II IGF receptor is identical to the mannose-6-phosphate receptor, which shuttles IGF-II (and lysosomal enzymes) from the cell surface, participating in IGF-II turnover. The type II receptor also mediates endothelial cell migration and induces neovascularization in the rat cornea. This receptor has signaling properties via cGMP, and in pancreatic cells mediates insulin exocytosis.

The six high-affinity IGFBPs bind IGFs with affinities in the range of $10^{-11}$ to $10^{-12}$ M and generally inhibit IGF availability to their receptors in target cells. Posttranslational IGFBP processing by proteolysis and glycosylation results in lowered IGFBP affinities for the IGFs, thereby increasing IGF availability to their receptors. IGFBP phosphorylation increases affinity for the IGFs and limits their access to their receptors. A family of IGFBP proteases has been identified which are metal-dependent enzymes of the kallikrein, cathepsin, matrix metalloproteinase, and disintegrin metalloproteinase families. These enzymes have unique specificities for the IGFBPs, although IGFBP-1 is the most resistant to proteolysis. IGFBP-1 and IGFBP-3 also have IGF-independent actions, by binding to cell surfaces and affecting cellular motility and cellular mitosis, respectively. Recently, four IGFBP-related proteins (IGFBP-rP-1-4) have been described that bind IGFs with low affinities and uniquely bind insulin. Their roles in IGF and insulin regulation await further investigation.

**THE IGF FAMILY IN CYCLING ENDOMETRIUM**

IGF peptides, IGFBPs, IGF receptors, and IGFBP proteases undergo unique changes during the menstrual cycle and in response to different hormonal stimuli of endometrial cells in vitro, suggesting unique functions for them under different steroid hormone conditions (Fig. 1). IGF-I mRNA is preferentially expressed in mid-late proliferative and early secretory endometrium, and IGF-II mRNA is expressed preferentially in secretory endometrium. Because of its temporal expression, IGF-I is believed to mediate the mitotic actions of E2 in this tissue. Indeed, IGF-I is stimulated by estradiol in endometrium of several species, including rodents, farm animals, and nonhuman primates. For example, high levels of IGF-I mRNA are expressed in rat uterus, which increases 20-fold in response to exogenously administered E2. IGF-I has also been implicated in estrogen-dependent neoplasms of human endometrium. Thus, IGF-I is believed to be one of several mediators in mitogenic actions of estradiol to effect rapid endometrial growth. IGF-II, expressed abundantly in midlate secretory endometrium is believed to be a mediator of progesterone action.

The type I IGF receptor is preferentially expressed in glandular epithelium with lower amounts in stroma. Since the IGF peptides are stromally derived, they likely participate in proliferation and cell survival of stroma and epithelium, by autocrine and paracrine mechanisms, respectively. IGF-I and IGF-II are mitogenic to in vitro cultured human endometrial cells and regulate secretory functions of stromal cells, supporting roles for these peptides in endometrium in vivo. IGFBP-1–6 mRNAs are expressed in human endometrium, primarily in stroma. IGFBP-5 is the only IGFBP that is expressed preferentially in proliferative phase endometrium. It is found in the ex-
tracellular matrix of many tissues, where it is proteolyzed to release IGFs for local tissue action. By analogy, IGFBP-5 may facilitate transport of the IGFs from their sites of synthesis to their sites of action during the proliferative phase of the cycle, although this has not been experimentally demonstrated. IGFBP-2, -3, -4, and -6 are differentially expressed in secretory phase endometrium where they likely regulate IGF actions locally. IGFBP-1 is the most abundantly expressed IGFBP and is a major protein product of secretory endometrium. It probably plays a major role in regulating IGF availability to receptors on both glandular epithelium and stroma during the secretory phase. In an IGFBP-1 transgenic mouse model, IGFBP-1 was abundantly expressed in the glandular epithelium, and impaired E2 actions on uterine DNA synthesis, compared to wild-type controls, suggesting that IGF-I is a mediator of E2 action in the rodent uterus.

IGFBP-4 protease activity has been identified in medium conditioned by human endometrial stromal cells decidualized in vitro. It is a metal-dependent enzyme whose activity is also IGF-dependent. As with other IGFBP proteases, it likely keeps IGF availability to receptors in equilibrium. However, the in vivo production of IGFBP-4 protease activity and its physiological significance remain to be determined.

Human endometrial stromal cells decidualized in vitro also produce IGFBP-rP-1. This protein is regulated (inhibited) by IGFs. The specificity of the production in endometrium is underscored by a lack of production of this protein by human cytotrophoblasts. Since the role of IGFBP-rPs is currently evolving, it will be of interest to see if IGFBP-rP-1 has a role in regulation of IGF or insulin action in the endometrium.

THE IGF FAMILY IN THE DECIDUAL: TROPHOBLAST INTERFACE

IGF-II mRNA is expressed in secretory endometrium and in uterine decidua from ectopic pregnancies. However, IGF-II is not expressed in decidua of intrauterine gestations, but rather is exclusively expressed in the placenta. Abundant IGF-II expression has been noted in the columns of the intermediate (invading) trophoblasts in the anchoring villi. There is a gradient of IGF-II mRNA expression in the columns, with greatest levels expressed at the invading front, suggesting a role for IGF-II in trophoblast invasion (Fig. 2).

IGF receptor mRNAs are expressed in placental trophoblasts, suggesting that these cells are targets for IGF action. IGF-I inhibits aromatase activity in trophoblasts in suspension culture. It also regulates glucose and amino acid transport in first trimester human placental trophoblasts. In addition, IGF-I stimulates hCG production in choriocarcinoma cells. These studies suggest that IGFs may play a role in trophoblast physiology and metabolism. In addition, the type II IGF receptor mediates the angiogenic actions of proliferin in rat cornea and stimulates endothelial cell migration in vitro. Whether IGF-II acting via the type II receptor is an angiogenic factor at the maternal:fetal interface or whether it is a mitogen, survival factor, or a regulator of trophoblast physiology remains to be determined.

IGFBPs in nonpregnant secretory phase endometrium are considerably upregulated in decidu-
ualized stromal cells, where they likely regulate IGF actions. The role of IGFBP-1 in implantation is considered in the next section.

Studies with cytotrophoblast monolayer cultures and with decidualized endometrial stromal cells in culture demonstrate that the cytotrophoblast secretes an IGFBP-3 protease. This protease decreases the affinity of IGFBP-3 for radio-labeled IGF-II > IGF-I and increases IGF bioavailability believed to be important in maternal tissue growth and perhaps placental growth. In clinical situations of fetal growth restriction lower levels of IGFBP-3 protease have been reported, and elevated levels have been observed in multiple gestations. These observations are consistent with the placenta being the origin of the IGFBP-3 protease. This protease has recently been identified as a member of the disintegrin metalloproteinase (ADAM) family.

IGFBP-1 AND IMPLANTATION

IGFBP-1, also known as placental protein-12 and α₁-progesterone-dependent endometrial globulin (α₁-PEG) is not a placental product, but rather is exclusively made in endometrium of a variety of species. In humans it is a major product of late secretory endometrium (16 µg/g protein), and is abundantly produced by maternal decidua, reaching levels of 1224 µg/g protein by midgestation, with concomitant marked induction of its mRNA. Immunoreactive IGFBP-1 localizes to the extracellular matrix and stromal cells of decidualizing endometrium, in the periarteriolar regions, and on the villous trophoblast, but not on placental fibroblasts. During the invasive phase of implantation, the "intermediate" trophoblast of the anchoring villous produces large amounts of IGF-II and invades into the maternal decidua stroma which is producing large amounts of IGFBP-1 and IGFBP-2 (Fig. 2). The spatial pattern and relative abundance of IGFBP-1 in decidua suggest it interacts with the IGF-II-expressing, invading trophoblast. IGFBP-1 has been shown to increase cytostrophoblast motility, and although its precise role in trophoblast invasion is not known, its abundance at the invading front and the proximity of decidual cells expressing IGFBP-1 are suggestive of a role for this growth factor and its inhibitor in invasion. IGFBP-1 has been shown to have inhibitory effects on IGF binding and IGF actions on choriocarcinoma cells in culture, and may have similar effects on IGF-II on normal, invading cytotrophoblasts at the maternal:fetal interface in vivo.

IGFBP-1 also has IGF-independent actions, by binding to cell membranes and altering cellular motility. IGFBP-1 contains the Arg-Gly-Asp (RGD) motif which is a recognition site for several cell adhesion molecules, including the α5β1 integrin. IGFBP-1 binds to the α5β1 integrin, presumably via its RGD sequence, and stimulates motility of Chinese Hamster Ovary (CHO) cells in vitro. In humans the invading trophoblast at the maternal:fetal interface uniquely expresses the α5β1 integrin, among all trophoblast phenotypes. Recent studies demonstrated that IGFBP-1 specifically binds to human trophoblasts and that it binds to the α5β1 integrin in trophoblast membranes. Furthermore, it inhibits trophoblast attachment to fibronectin, another RGD ligand found in the placental bed. Cytotrophoblast interaction with fibronectin, through the α5β1 integrin, restrains invasion. Human trophoblasts do not invade into human endometrial stromal cell multilayer cultures decidualized in vitro, which produce high levels of IGFBP-1. However, when IGFBP-1 production by the stromal cells is inhibited by insulin, it results in trophoblast invasion into the stromal multilayers. Furthermore, invasion is inhibited by the addition of exogenous IGFBP-1 into the coculture system, in a dose-dependent fashion. These studies cumulatively suggest that IGFBP-1 is a maternal "restraint" on trophoblast invasion. Whether it does this via direct interactions with the trophoblast (as an α5β1 ligand or a ligand for other cell surface proteins) or by inhibiting IGF-II actions on the trophoblast is not known at this time. The role of IGFBP-1 in implantation, however, is currently controversial, since it has also been shown to stimulate migration of passaged human trophoblasts across a Matrigel extracellular matrix barrier in the presence of serum. In this experimental system IGF-II is also a stimulator of passaged trophoblast migration and invasion. In primary trophoblast cultures, IGFBP-1 increases total gelatinase activity, suggesting it enhances trophoblast invasiveness. However, the specific enzyme(s) and/or inhibitors/activators responsible for this rise in gelatinase activity remained unidentified, since IGFBP-1 showed no effect on MMP-2 or MMP-9 and increased TIMP-1 in these studies. These seemingly conflicting observations likely depend on different experimental paradigms, cell populations, and endpoints for evaluating IGFBP-1 function. The role(s) of IGFBP-1, either as a direct modulator of trophoblast invasion or as an IGF-II binding protein, at the maternal:fetal interface should be forthcoming in the near future.
REGULATION OF IGFBP-1 IN ENDOMETRIUM

In vivo human endometrial stromal cells, after estrogen-priming, undergo proliferation and differentiation in response to progesterone. Endometrial stromal cells can be decidualized in vitro with progesterone or progestins, or stimulators of cyclic AMP (cAMP), and estradiol and epidermal growth factor (EGF). This in vitro model has provided an opportunity to investigate production and regulation of IGFBP-1, which is produced primarily by this cell type in endometrium in vivo. IGFBP production in endometrium is dependent on stromal differentiation and, while decidualized stromal cells increase their production of all IGFBPs upon decidualization in vivo, most striking are the high levels of IGFBP-1 (25 µg per day per 10^6 cells), in contrast to another decidual marker, prolactin (40 ng per 10^6 cells per day). Progesterone regulates IGFBP-1 protein and mRNA expression in decidualized endometrial stromal cells, and the progesterone receptor antagonist RU486 is inhibitory. The IGFBP-1 gene has a glucocorticoid response element and a steroid hormone response element, and it is likely that progesterone exerts its effects on IGFBP-1 gene expression via these promoter elements.

Insulinlike Peptides

The close proximity of the IGF-II-producing trophoblast and the IGFBP-1-producing decidua suggests either an effect of IGF-II on the trophoblast and/or an effect of IGF-II on decidual (and/or trophoblast) function. Insulin and IGFs are known inhibitors of IGFBP-1 in liver and HepG2 cells. Similarly, in endometrium, insulin, IGF-I, and IGF-II inhibit decidualizing stromal cell IGFBP-1 secretion into conditioned media (CM), in a dose-dependent fashion, with ED_50's consistent with action through their cognate receptors. Insulinlike peptides are also inhibitory to IGFBP-1 production by endometrial stromal cells decidualized in vivo. The physiologic relevance of IGF regulation of IGFBP-1, a major product of secretory phase endometrium and decidua, likely rests on the need to regulate IGF availability to target cells, including trophoblast and/or decidua and endometrial glandular epithelium. In addition, these regulatory mechanisms may affect direct interactions of this IGFBP with the invading trophoblast (see above). However, the physiologic relevance of insulin regulation of IGFBP-1 production by endometrial stromal cells or decidua is unresolved. In contrast to minute-to-minute changes in insulin and IGFBP-1 in the circulation, it is unlikely that there are wildly fluctuating levels of IGFBP-1 at the decidual-trophoblast interface throughout the day. How insulin action is controlled at the level of the endometrium remains unresolved, although IGFBP-rPs may be involved, as well as insulinases, and other modulators of insulin action. This question is particularly relevant for women with anovulatory infertility who have insulin resistance and hyperinsulinemia, as with polycystic ovary syndrome.

Cytokines, Growth Factors, and Chorionic Gonadotropin

The question naturally arises as to whether other growth factors and cytokines at the decidua: trophoblast interface regulate IGFBP-1 production in the maternal compartment. A recent study investigated the effects of several cytotrophoblast products on endometrial stromal IGFBP-1 production in vitro, including interleukin-1β (IL-1β), transforming growth factor-β (TGF-β), stem cell factor (SCF), colony stimulating factor-1 (CSF-1), leukemia inhibitory factor (LIF), and IGF-II. Only IGF-II and IL-1β had an effect; both were inhibitory, with ED_50's consistent with actions through their cognate receptors. IL-1β has also inhibits the process of decidualization of endometrial stromal cells. Since IL-1β stimulates production of trophoblast matrix metalloproteinase-9 (MMP-9) which promotes the invasive trophoblast phenotype, it is likely that inhibition of IGFBP-1 in the decidua by this cytokine further promotes trophoblast invasion, by inhibiting this decidual "maternal restraint protein" (see below).

Human chorionic gonadotropin (hCG) stimulates endometrial stromal IGFBP-1 production, likely due to its effects on promoting stromal decidualization. Similar results have been reported with free hCG-α subunit, although the mechanisms for free α-subunit action are not well understood. However, hCG has no appreciable effect on stromal cell products once the cells are decidualized in vivo. While the physiologic relevance of hCG action in endometrium is not well established, a premature luteinizing hormone (LH) surge may lead to premature decidualization of the endometrium (via untimely production of progesterone). Since elevated LH has been associated with an increased risk of spontaneous abortion, whether elevated endometrial IGFBP-1 predisposes to poor implantation and miscarriage is an important clinical issue which remains to be resolved. Since IL-1β, IGF-II, and hCG are trophoblast-derived, these observations cumulatively support roles for select trophoblast-derived growth factors and cytokines in
the regulation of decidualization of the maternal endometrium and of maternally-derived IGFBP-1 at the trophoblast:decidual interface (Fig. 2). This dialogue between the trophoblast and endometrium is likely to have importance in normal as well as abnormal placentation.

**Hypoxia**

In pregnancies complicated by uteroplacental insufficiency, maternal and fetal circulating IGFBP-1 levels are markedly elevated. Recently, our group has demonstrated that the IGFBP-1 gene has a hypoxia response element in intron 1. Regulation of this response by hypoxia was confirmed using a human hepatoma cell line, and this response is augmented in the presence of cAMP. Preliminary studies indicate that endometrial stromal cells, decidualized in vitro, respond to a chemical hypoxicant (CoCl₂) with a twofold increase in IGFBP-1 mRNA and a four- to sixfold increase in protein. An elevation of IGFBP-1 at the decidua: trophoblast interface could result in shallow implantation, which could greatly compromise nutrient and oxygen transfer to the placenta and fetus, resulting in fetal growth restriction (also see below).

**IGFBP-1 IN A HUMAN PREGNANCY DISORDER OF SHALLOW IMPLANTATION**

Preeclampsia, a disorder specific to pregnancy, occurs in 5–10% of pregnant women, and in its severe form is a major cause of fetal and maternal morbidity and mortality. Clinically, it is usually detected in the third trimester, and if left undiagnosed or untreated, can progress to maternal multiorgan failure, coagulopathy, seizures, and maternal and fetal death. In the severe form of the disorder there is generalized hypoxia within the placenta and decidua, and abnormally shallow cytotrophoblast invasion into the uterine decidua. The latter is believed to result from abnormal trophoblast adhesion molecules and/or from elevated decidual levels of IGFBP-1 preventing deeper trophoblast invasion. In support of the latter hypothesis is the clinical finding that in pregnancies complicated by severe preeclampsia, maternal serum IGFBP-1 levels in the second and early third trimesters are about sixfold higher and at term are about twofold higher than in normal pregnancies. This is not a nonspecific elevation of hepatic-derived proteins, since maternal serum levels of IGFBP-3 (also of hepatic origin) are normal and IGFBP-3 is not a useful predictor of preeclampsia. In addition, hepatic-derived IGF-I levels in the circulation are about half of control levels. A significant correlation was observed between maternal diastolic blood pressure, aspartate transcarbamylase and IGFBP-1, suggesting that IGFBP-1 reflects severity of preeclampsia and hepatic involvement. It is likely that elevated levels of IGFBP-1 in the circulation of women with severe preeclampsia derive from the liver and decidua. In decidua IGFBP-1 is found in the periarteriolar region. With enhanced vascular permeability and vasospassm, common in preeclampsia, escape from the decidua into the maternal circulation may occur, contributing to the observed elevated serum levels of this binding protein in this disorder. Higher levels of immunoreactive IGFBP-1 were observed at the decidua:placental interface in pregnancies complicated by severe preeclampsia, compared to controls. Recent preliminary observations of hypoxic induction of IGFBP-1 in the decidua (see above) support the hypothesis that IGFBP-1 is a maternal restraint protein that is part of the pathophysiology of preeclampsia. However, the decidual origin of the elevated IGFBP-1 at the decidua:placental interface and in the maternal circulation in severe preeclampsia remains to be determined. In a recent longitudinal study, IGFBP-1 levels were found to be decreased in maternal serum in midgestation in women who subsequently developed mild preeclampsia. It is likely that mild and severe preeclampsia are different disorders, and controversy still surrounds whether circulating IGFBP-1 early in gestation is a predictor of development of the more fulminant form of the disease later in gestation.

**SUMMARY**

IGFs, their binding proteins, binding protein peptides, and receptors are complex participants in regulating cellular mitosis, survival, metabolism, motility, and migration. Endometrium is a dynamic tissue in which cyclic, steroid-dependence of these processes occurs. In addition, their participation at the decidual: trophoblast interface appears to be important with regard to decidual and trophoblast function and physiology. While there are still gaps to fill in understanding the precise roles of some of the members of the IGF family in endometrial, decidual, and trophoblast cellular function, determining their roles in abnormal endometrial development and abnormal implantation remains a challenge for the future.

**REFERENCES**


10. Murphy LJ, Murphy LC, Friesen HG. A role for the insulin-like growth factors as estrogens in the rat uterus. Trans Assoc Am Physicians 1987;59:204–214


24. Murphy LJ, Murphy LC, Friesen HG. A role for the insulin-like growth factors as estrogens in the rat uterus. Trans Assoc Am Physicians 1987;59:204–214


38. Langford KS, Nicolaides KH, Jones J, Abbass A, McGregor AM, Miell JP. Serum insulin-like growth factor-binding...
56. Jones JL, Cockerman A, Busby WH, Clemons DR. IGFBP-1 stimulates cell migration and binds to the a5b1 integrin EGF and epidermal growth factor receptor sequences. Proc Natl Acad Sci USA 1993;90:10553–10556
58. Irwin JC, Giudice LC. IGFBP-1 binds to the cytotrophoblast a5b1 integrin and inhibits cytotrophoblast invasion into decidual multilayers. Growth Horm IGF Res 1998;8:21–31
74. Li TC, Serle E, Warren MA, Cooke ID. Is endometrial development in the perimplantation period influenced by high concentrations of LH in the follicular phase? Hum Reprod 1993;8:1021–1024
76. Howell RJS, Perry LA, Choglay NS, Bohn H, Chard T. Placental protein-12 (PP12): A new test for the prediction of...
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88. Than GN, Csaba IF, Szabo DG, Arany AA, Bognar ZJ, Bohn H. Serum levels of placental-specific tissue protein 12 (PP12) in pregnancy complicated by pre-eclampsia, diabetes, or twins. Arch Gynecol 1984;236:41–45


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