Complex mediation of uterine endometrial epithelial cell growth by insulin-like growth factor-II (IGF-II) and IGF-binding protein-2

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ABSTRACT

The coexpression of IGF (-I and -II) peptides, corresponding receptors, and IGF binding proteins (IGFBPs) in uterine endometrium suggests that a significant component of IGF action in this tissue is via autocrine or paracrine pathways, or both. The present study examined whether IGF-II and a major uterine-expressed IGF-II binding protein, IGFBP-2, modulate endometrial epithelial cell mitogenesis. Serum-deprived porcine endometrial glandular epithelial (GE) cells of early pregnancy were treated with various concentrations of IGFs, recombinant porcine (rp) IGFBP-2, or both, and examined for changes in cellular mitogenesis by incorporation of [³H]thymidine into DNA. Recombinant human (rh) IGF-II stimulated DNA synthesis in a dose-dependent manner. Human [Leu²⁷]-IGF-II, an analog with selective affinity for the IGF-II (type II) receptor, increased thymidine uptake by twofold compared with untreated GE cells. When added in combination with an equimolar concentration of rhIGF-I, [Leu²⁷]-IGF-II or rhIGF-II stimulated thymidine incorporation to a

greater extent than did rhIGF-I alone. Ligand blot analysis of GE cell conditioned medium revealed the presence of four IGFBPs with molecular masses of 48, 31, 23, and 15 kDa. Physiological concentrations of rpIGFBP-2 (nM range) increased both basal and IGF-induced DNA synthesis in GE cells. At equimolar concentrations, Des(1-6)IGF-II (an IGF-II analog with much reduced affinity for IGFBPs) and rpIGFBP-2 had additive effects on GE cell mitogenesis, suggesting that the IGFBP-2 modulation of uterine cell growth may involve both IGF-dependent and IGF-independent pathways. Our results demonstrate the complex interplay of IGF system components in uterine endometrial epithelial growth regulation *in vitro*, identify IGF-II and IGFBP-2 as locally coexpressed uterine epithelial cell mitogens, and suggest the presence of a functional signaling pathway by which IGF-II stimulates epithelial cell proliferation via the type II receptor.

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INTRODUCTION

The uterus undergoes hyperplasia in addition to morphological and functional differentiation during estrous cycles and pregnancy. These physiological states are characterized by highly coordinated changes in endometrial and myometrial mRNA

expression of insulin-like growth factors (IGFs), IGF receptors and IGF binding proteins (IGFBPs) – the IGF system. In the uterus of several species, including the pig and rat, IGF-I mRNAs predominate in early pregnancy, whereas IGF-II mRNA accumulation occurs primarily after implantation (Letcher *et al.* 1989, Pescovitz *et al.* 1991, Simmen

et al. 1992), suggesting that these mitogens have either distinct or overlapping actions at the embryo–maternal and feto–maternal interfaces, respectively. The high peri-implantation expression of the *IGF-I* gene in porcine uterus coincides temporally with increased uterine luminal fluid IGF-I content (Simmen *et al.* 1989), elongation of spherical blastocysts to the filamentous morphology (Geisert *et al.* 1982), and transient conceptus secretion of estrogens (Gadsby *et al.* 1980, Pusateri *et al.* 1990, Green *et al.* 1995), which are paracrine regulators of endometrial function, possibly in concert with endometrial-synthesized IGF-I. Because uterine endometrium and myometrium manifest relatively high levels of IGF receptors (Ghahary & Murphy 1989, Talavera *et al.* 1990, Hofig *et al.* 1991), it is speculated that these peptide ligands constitute important autocrine/paracrine effectors of coordinate uterine and embryonic development.

In most of the tissue and cell systems examined to date, the mitogenic actions of IGF-I and IGF-II are considered to be mediated by the IGF-I (type I) receptor (reviewed in Jones & Clemmons 1995). The role of the IGF-II/cation-independent mannose-6-phosphate (type II) receptor in IGF-II signaling is less clear, and somewhat controversial. Previous attempts to examine which IGF receptor subtype mediates a specific effect(s) of IGF have used anti-receptor antibodies (Jones & Clemmons 1995). However, under certain experimental conditions, these antibodies do not result in either complete or partial inhibition of binding (Casella *et al.* 1986, Misra *et al.* 1986, Kojima *et al.* 1988, Steele-Perkins & Roth 1990) and, instead, appear to function as IGF receptor agonists (Kojima *et al.* 1988, Steele-Perkins *et al.* 1988). The recent availability of human [Leu²⁷]-IGF-II, an analog with highly selective affinity for the IGF-II receptor (Beukers *et al.* 1991, Bürgisser *et al.* 1991, Sakano *et al.* 1991), has allowed re-examination of the IGF receptor subtype that mediates IGF-II-induced cell mitogenesis.

In addition to their receptors, the biological actions of IGFs also are modulated by a family of structurally related IGFBPs, present in many biological fluids, cell cultures and tissues (Rechler 1993, Jones & Clemmons 1995). These proteins share the ability to bind IGF-I and IGF-II with high affinity, and often are associated with cell membranes, extracellular matrix, or both, where they may influence IGF–IGF receptor interactions and possibly exert IGF-independent functions (Rechler 1993, Jones & Clemmons 1995). Although the complete mechanism(s) of action has not been fully defined for any of the IGFBPs, these proteins

can augment or inhibit IGF action, depending upon the nature of the IGFBP, the cell context and the experimental conditions. Uterine endometrial expression of IGFBP-2 has been demonstrated in the human (Giudice *et al.* 1991), pig (Simmen *et al.* 1992, Song *et al.* 1996), and rat (Cerro & Pintar 1997), in addition to other species. In pregnant pig uterus, the abundance of IGFBP-2 mRNA and protein is induced during late pre-implantation (around day 15, postestrus) and reaches maximal levels by mid-pregnancy (day 60) (Simmen *et al.* 1992, Song *et al.* 1996). This temporal expression pattern mimics that for endometrial epithelial IGF-II (Simmen *et al.* 1992), and implicates a physiological role(s) for IGFBP-2 and IGF-II in implantation events and subsequent feto–maternal interactions. However, the biological nature of these presumed roles and the functional interdependency of the two proteins remain unknown for any species.

In the present study, we have examined the individual effects and potential functional interactions of IGF-II and IGFBP-2 on uterine epithelial cellular mitogenesis, using primary cultures of porcine uterine endometrial glandular epithelial (GE) cells of early pregnancy (day 12, postestrus), which express low amounts of endogenous IGFBP-2 relative to later pregnancy stages. Here, we demonstrate the stimulatory effects of IGFBP-2 on basal and exogenous IGF-II-induced DNA synthesis in normal endometrial epithelial cells and begin to clarify the pathway(s) through which IGF-II and IGFBP-2 modulate endometrial epithelial cell proliferation.

MATERIALS AND METHODS

Materials

Recombinant human (rh) IGF-I and rhIGF-II peptides were purchased from Upstate Biotechnology (UBI, Lake Placid, NY, USA). [Leu²⁷]-IGF-II and Des(1–6)IGF-II were obtained from GroPep Pty, Ltd (North Adelaide, Australia). Cell culture media and TRIzol were from Gibco-Brl (Gaithersburg, MD, USA). Antibiotic–antimycotic solution (ABAM) and fetal bovine serum (FBS) were from Sigma Chemical Co. (St Louis, MO, USA). [³H]Thymidine (specific activity 6.7 Ci/mmol), [α -³²P]deoxycytidine triphosphate (specific activity 3000 Ci/mmol) and BioTrans nylon membranes (0.2 μ m) were purchased from ICN Pharmaceuticals (Irvine, CA, USA). Nitrocellulose membranes (0.2 μ m) were obtained from Schleicher and Schuell (Keene, NH, USA).

FIGURE 1. Bacterial expression construct for rpIGFBP-2. A construct consisting of IGFBP-2 cDNA in the pET-15b expression vector was used to produce recombinant protein in *E. coli*. The rpIGFBP-2 was purified by His-bind affinity chromatography followed by non-denaturing preparative gel electrophoresis and electro-elution.

Recombinant porcine (rp) IGFBP-2

A porcine thoracic aorta muscle cDNA library in pBluescript SK(-) vector was screened with a human IGFBP-2 cDNA probe to isolate several cDNA clones, which, by sequence analysis, were confirmed to encode porcine IGFBP-2 (GenBank AF120326). A cDNA fragment encoding the mature form (i.e. missing the signal peptide) of porcine IGFBP-2 was amplified from the cloned cDNA by Expand high-fidelity PCR using 30-mer oligodeoxynucleotides as primers (forward: 5-AGC CATATGGAGGTGCTGTTCCGCTGCCCG-3; reverse: 5-TTGCTCAGCCTGCATCCGCTGG GTGTGTGC-3). The PCR was performed in 50 µl buffer (Buffer C, PCR Optimizer Kit, Invitrogen Corp., Carlsbad, CA, USA) and containing 50 ng template, 200 ng each primer, 250 µM dNTPs, and 10% dimethyl sulfoxide. The PCR products were ligated to pCARTII vector (TA cloning Kit, Invitrogen) and recombinant clones were selected after transformation of BL21(DE3) *Escherichia coli* competent cells. The IGFBP-2 cDNA inserts were released from three such clones and individually subcloned between the unique NdeI and BspI sites of the pET-15b vector (Novagen, Madison, WI, USA). The three resultant IGFBP-2-pET-15b expression constructs (Fig. 1) were then used to transform the BL21(DE3) strain

of *E. coli* and sequenced in their entirety to confirm identity to wild-type sequence. Bacterial cells were grown to an OD₆₀₀ of 0.6 in Luria-Bertani medium containing 100 µg/ml ampicillin, at which point, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM, to induce synthesis of IGFBP-2. Cells were grown for an additional 2 h, and then centrifuged at 800 *g* for 10 min. Cell pellets were resuspended in binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris, pH 7.9) and sonicated on ice using five 2–3 s bursts. Sonicates were centrifuged at 47 000 *g* for 30 min at 4 °C and the supernatants and pellets (inclusion bodies) were separately collected. The rpIGFBP-2, which contains a hexa-histidine motif within its amino-terminal extension (Fig. 1), was purified by affinity chromatography on His-bind resin (Qiagen Inc., Valencia, CA, USA), followed by non-denaturing preparative PAGE and electro-elution.

Cell culture

Uterine endometrial GE cells were isolated from day 12 pregnant pig endometrium as previously described (Reed *et al.* 1996). All animal-use procedures were approved by the University of Florida Institutional Animal Care and Use Committee. Cells were resuspended (0.5×10^6 cells/ml)

in RPMI 1640 medium containing 10% FBS, 1% ABAM and 0.25 U/ml insulin and cultured at 37 °C in a 95% air–5% CO₂ environment. Cells remained undisturbed (without medium change) for the first 3 days after plating. Thereafter, each culture was replenished with fresh medium every 2 days until cells reached the desired cell density.

[³H]Thymidine incorporation assay

Mitogenic effects of recombinant IGF-II, IGF-II analogs and IGFBP-2 were examined via the incorporation of [³H]thymidine into DNA of serum-deprived, confluent GE cells. Cells were seeded in six-well plates and grown to confluence at 37 °C. Cultures were then washed twice with Hanks' balanced salt solution (HBSS) and preconditioned in serum-free medium (containing ABAM) for 24 h. Medium was changed to fresh (serum-free) medium and the cells incubated with or without the indicated treatments for 24 h. After 20 h, cells were pulse-labeled with 2 µCi [³H]thymidine for 4 h. Cells were rinsed with phosphate-buffered saline (pH 7.4), DNA was precipitated with 5% trichloroacetic acid, and incorporated radioactivity was measured by liquid scintillation counting.

Ligand blot analysis

Confluent GE cells were washed twice with HBSS and then preconditioned in serum-free medium for 24 h. Medium was then removed and the cells rinsed and incubated in fresh serum-free medium for an additional 24 h. Conditioned medium (CM) was collected after the second incubation and centrifuged briefly to remove cells and insoluble material. CM proteins were lyophilized and subjected to 12.5% SDS-PAGE under non-reducing conditions, followed by electro-transfer to nitrocellulose membrane. The filters were blocked with Tris–buffered saline (TBS, pH 7.4) containing 1% Carnation non-fat dry milk and incubated with 4 × 10⁶ c.p.m. [¹²⁵I]-labeled rhIGF-II in this same buffer for 24 h at 4 °C. After several washes with TBS, the air-dried membranes were exposed to BioMax film (Eastman Kodak Co, Rochester, NY, USA) for 24 to 48 h at –80 °C.

Statistical analysis

Differences in [³H]thymidine incorporation attributable to treatment were evaluated by least-squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS 1985). When significant effects were detected ($P < 0.05$), means were separated by preplanned

FIGURE 2. Electrophoretic analysis of rpIGFBP-2. A, Purified rpIGFBP-2 was resolved by 12.5% SDS-PAGE under reducing conditions and stained with Coomassie Blue. B, Increasing amounts of purified rpIGFBP-2 were resolved in a 12.5% SDS-PAGE under non-reducing conditions, electro-blotted to a nitrocellulose membrane, probed with [¹²⁵I]rhIGF-II, and visualized by autoradiography. The migration positions of pre-stained protein standards are indicated on the left.

orthogonal contrasts. Data are presented as means ± S.E.M. of three independent experiments.

RESULTS

Bacterial expression and functional analysis of rpIGFBP-2

In the pig uterine endometrium, IGFBP-2 mRNA abundance is highly induced during peri-implantation (around day 15, post-estrus) and reaches maximal levels by mid-pregnancy (Simmen *et al.* 1992, Song *et al.* 1996). This temporal expression pattern points to a physiological role for IGFBP-2 in implantation and subsequent fetomaternal interactions. To examine how this protein might interact with and affect uterine cell function, a construct consisting of pIGFBP-2 cDNA in pET-15b expression vector was generated and used to produce recombinant protein in *E. coli* (Figs 1, 2). Consistent with the size of porcine IGFBP-2 (Lee *et al.* 1991), the measured size of rpIGFBP-2 fused to the amino-terminal extension containing a hexa-histidine motif was 36 kDa (Fig. 2). The crude recombinant protein was isolated predominantly from the supernatant fraction, rather than from the insoluble inclusion bodies (data not shown). The recombinant protein used for all experiments was subsequently purified to near homogeneity by His-bind affinity chromatography followed by

FIGURE 3. IGFBPs secreted by porcine uterine endometrial cells. CM proteins prepared from confluent monolayers of (A) human Hec-1A cells (positive control for IGFBP-2 and IGFBP-3) and (B) porcine GE cells were separated by 12.5% SDS-PAGE under non-reducing conditions. The proteins were electro-blotted to nitrocellulose membrane, probed with [125 I]rhIGF-II, and visualized by autoradiography. The two bands (in descending molecular mass) for Hec-1A CM represent IGFBP-3 and IGFBP-2. Note the absence of IGFBP-2 in GE cell CM. The migration positions of pre-stained protein standards are indicated on the left.

non-denaturing preparative gel electrophoresis-electro-elution and was not treated with thrombin. Ligand blot analysis of the purified protein with radio-labeled rhIGF-II revealed a sharp band that migrated at 36 kDa, demonstrating its functional IGF-binding activity (Fig. 2).

IGFBPs in GE cell conditioned medium

The presence and identities of IGFBPs in GE cell CM were examined by ligand blotting using [125 I]-labeled rhIGF-II. Four IGFBP bands with molecular masses of 48, 31, 23, and 15 kDa were detected in the GE cell CM (Fig. 3). IGFBP-2 (34 kDa protein) was not detected in GE cell CM by either ligand blot (Fig. 3) or Western blot analyses (data not shown). The highest-molecular-weight protein in GE CM appeared as a diffuse band between 53 and 45 kDa, which is consistent with the size of IGFBP-3 (Lee *et al.* 1991, Ko *et al.* 1994).

FIGURE 4. Mitogenic response of uterine endometrial GE cells to IGF-II. GE cells were grown to confluence and then incubated in serum-free medium for 24 h. The cell monolayers were rinsed with HBSS and incubated with increasing concentrations of rhIGF-II in serum-free medium for an additional 24 h. After 20 h of this incubation, cells were pulse-labeled with 2 μ Ci [3 H]thymidine for 4 h. The mitogenic response to each treatment was determined in triplicate and results represent the mean $\pm$ S.E.M. of three independent experiments for each rhIGF-II concentration. Each experiment represents cells isolated from an individual pig on day 12 of pregnancy. Means with differing superscripts are statistically significantly different ($P < 0.05$).

Mitogenic effects of IGF peptides in GE cells

To examine the role of IGF-II in uterine cell growth, serum-deprived GE cells were treated with various concentrations of rhIGF-II and its analog, [Leu 27]-IGF-II, for 20 h and pulsed with [3 H]thymidine for 4 h. IGF-II stimulated DNA synthesis in porcine endometrial GE cells in a dose-dependent fashion (Fig. 4). [Leu 27]-IGF-II, an analog with highly selective affinity for the IGF-II receptor (little or no binding to the type I receptor), increased mitogenesis of GE cells to the same extent as did native IGF-II (Fig. 5). When added to the culture medium in combination with an equimolar concentration of rhIGF-I, native IGF-II and its analog stimulated [3 H]thymidine incorporation to a greater extent than did rhIGF-I alone (Fig. 6). These collective findings suggest the involvement of both IGF receptor types in uterine GE cell mitogenesis.

rpIGFBP-2 mediation of GE cell proliferation

To examine whether IGFBP-2 functionally interacts with IGF-II to mediate uterine epithelial cell

FIGURE 5. Effect of [Leu²⁷]-IGF-II on [³H]thymidine incorporation into DNA of porcine endometrial GE cells. Confluent monolayers of GE cells were pre-conditioned in serum-free medium for 24 h and then treated with increasing concentrations of [Leu²⁷]-IGF-II for an additional 24 h. After 20 h of this incubation, cells were pulse-labeled with 2 μ Ci [³H]thymidine for 4 h. The mitogenic response to each treatment was determined in triplicate and results represent the mean $\pm$ S.E.M. of three independent experiments for each concentration of the IGF-II analog. Each experiment represents endometrial GE cells isolated from an individual pig on day 12 of pregnancy. Means with different superscripts are statistically significantly different ($P < 0.05$).

growth, serum-deprived GE cells were incubated with various concentrations of rpIGFBP-2 in the presence or absence of exogenous IGF-II. Physiological doses (nM) of rpIGFBP-2 increased IGF-II-dependent DNA synthesis in primary cultures of GE cells (Fig. 7). These effects of rpIGFBP-2 on cellular DNA synthesis were also demonstrated in the absence of exogenous rhIGF-II (Fig. 8). Lastly, Des(1–6)IGF-II, an analog with markedly reduced affinity for IGFBPs, stimulated DNA synthesis by 1.5-fold in GE cells and cotreatment with an equimolar concentration of rpIGFBP-2 further increased Des(1–6)IGF-II-induced mitogenesis of these cells (Fig. 9).

DISCUSSION

The present study examined the potential involvement and functional interactions of IGF-II and a major uterine-expressed IGF-II binding protein,

FIGURE 6. Additive effects of IGF peptides on DNA synthesis of porcine endometrial cells. Confluent monolayers of GE cells were washed with HBSS and incubated in serum-free medium for 24 h. Cells were then incubated with or without the indicated peptides for an additional 24 h. After 20 h of this incubation, cells were pulse-labeled with 2 μ Ci [³H]thymidine for 4 h. Mitogenic responses were determined in triplicate and results represent the mean $\pm$ S.E.M. for three independent experiments. Each experiment represents cells isolated from an individual pig on day 12 of pregnancy. Means with different superscripts are statistically significantly different ($P < 0.05$).

IGFBP-2, in mediating uterine endometrial cell proliferation. Our results demonstrating the mitogenic activities of these proteins, alone and in combination, suggest important uterine roles *in vivo*, not otherwise demonstrated by the transgenic and knock-out methodologies used in studies of uterine IGF-I (Baker *et al.* 1996, Wang *et al.* 1997).

The presence of both IGF receptor types on most cells and the cross-reactivity of ligands for binding to these receptors have made it difficult to determine the specific receptor type that mediates a given biological response. Although previous studies suggested that cell signaling by both IGF-I and IGF-II is mediated primarily through the tyrosine kinase IGF-I receptor (reviewed in Jones & Clemmons 1995), other reports indicated that IGF-II may elicit some of its effects through the non-tyrosine kinase IGF-II-cation-independent mannose-6-phosphate receptor (Beguinot *et al.* 1985, Tally *et al.* 1987, Minniti *et al.* 1992, Takigawa *et al.* 1997). To understand further the mechanism by which IGF-II affects uterine function, we examined the IGF receptor subtype(s)

Treatment

FIGURE 7. Dose-dependent effect of rpIGFBP-2 on IGF-II-induced [³H]thymidine incorporation into DNA of uterine endometrial cells. Confluent monolayers of GE cells were incubated in serum-free medium for 24 h. Cells were rinsed and then incubated with rhIGF-II (5 nM) in the presence or absence of various concentrations of rpIGFBP-2 in serum-free medium for 24 h. After 20 h of this incubation, cells were pulse-labeled with 2 μ Ci [³H]thymidine for 4 h. The mitogenic response to each treatment combination was determined in triplicate and results represent the mean $\pm$ S.E.M. for three independent experiments (each experiment represents cells isolated from an individual pig on day 12 of pregnancy). Means with different superscripts are statistically significantly different ($P < 0.05$).

that mediates IGF-II-induced DNA synthesis in porcine endometrial GE cells, by means of [Leu²⁷]-IGF-II. This analog interacts with the IGF-II-mannose-6-phosphate receptor with normal affinity, but has negligible affinity for IGF-I and insulin receptors (Beukers *et al.* 1991, Bürgisser *et al.* 1991, Sakano *et al.* 1991). [Leu²⁷]-IGF-II increased DNA synthesis in a dose-dependent manner in porcine endometrial GE cells, and these stimulatory effects were detected at concentrations (nM range) at which the analog interacts only with the IGF-II receptor. Moreover, [Leu²⁷]-IGF-II or native IGF-II, in combination with equimolar concentrations of rhIGF-I, stimulated [³H]thymidine incorporation to greater extents than did IGF-I alone. These findings suggest the presence of functional IGF-II-mannose-6-phosphate receptors on porcine uterine epithelial cells, and point to the involvement of the type II IGF receptor, distinct from that of IGF-I, in mitogenic signaling of these cells.

Significant uterine endometrial IGFBP-2 expression has been demonstrated for a number of mammals (Giudice *et al.* 1991, Rechler 1993, Song

FIGURE 8. rpIGFBP-2 modulation of basal mitogenesis of uterine endometrial cells. Confluent monolayers of GE cells were incubated in serum-free medium for 24 h. Cells were rinsed with HBSS and further incubated in the presence of increasing concentrations of rpIGFBP-2 for 24 h. After 20 h of this incubation, cells were pulse-labeled with 2 μ Ci [³H]thymidine for 4 h. The mitogenic response to each treatment was monitored in triplicate and results shown are the mean $\pm$ S.E.M. for three independent experiments for each concentration of rpIGFBP-2. Means with different superscripts are statistically significantly different ($P < 0.05$).

FIGURE 9. Additive effects of Des(1-6)IGF-II and IGFBP-2 on [³H]thymidine incorporation by porcine endometrial cells. Responses were determined in triplicate during each of three independent experiments. Each experiment utilized cells from a different pig on day 12 of pregnancy. Means with different superscripts are statistically significantly different ($P < 0.05$).

et al. 1996, Cerro & Pinter 1997). In porcine uterus, this expression is induced during the peri-implantation period and reaches maximal levels by mid-pregnancy. In the present study, we sought to examine whether this binding protein interacts with its major ligand, IGF-II, to modulate uterine endometrial cell proliferation during early pregnancy. In contrast to its well-documented inhibitory effects on DNA synthesis in tumor cell lines (reviewed in Rechler 1993 and Jones & Clemmons 1995), rpIGFBP-2 increased both basal and exogenous IGF-II-stimulated [³H]thymidine incorporation in primary cultures of porcine endometrial epithelial cells. Consistent with these data, Bar *et al.* (1989) previously reported that partially purified IGFBP-2 was a weak potentiator of IGF-I-induced glucose transport and aminoisobutyric acid uptake in microvascular endothelial cells. Likewise, bovine IGFBP-2 was shown to potentiate the mitogenic response of aortic smooth muscle cells to IGF-I by 80% in the presence of low concentrations of platelet-poor plasma (Bourner *et al.* 1992). In addition, the homozygous IGFBP-2 null mutant mouse is characterized by decreased spleen size (Pinter *et al.* 1996), suggestive of a stimulatory role for this IGFBP in tissue growth. The observation that exogenous Des(1–6)IGF-II, an analog with minimal affinity for IGFBPs, and exogenous rpIGFBP-2 had additive effects on GE cell mitogenesis further suggests that IGFBP-2 modulation of uterine epithelial cell growth may involve both IGF-dependent and IGF-independent pathways.

In summary, these results demonstrate that IGFBP-2 and IGF-II are potent local mitogens for the uterine epithelium and that this effect of both proteins is, in all likelihood, not wholly mediated by the type I IGF receptor. These results are intriguing, in light of the presence of significant amounts in porcine uterus of truncated (Des)IGF-I (Ballard *et al.* 1996), which, together with IGF-II and IGFBP-2, might mediate proliferative and differentiative events in the endometrium via multiple parallel or convergent pathways.

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