Immunohistochemical Localization of the Insulinlike Growth Factor Binding Protein-1 in Female Reproductive Tissues by Monoclonal Antibodies

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Abstract

Human insulinlike growth factor binding protein-1 (hIGFBP-1) is a secretory protein that modulates the receptor-binding and biological actions of the insulinlike growth factor I (IGF-I). Human endometrium expresses the mRNA for IGFBP-1, and this protein is secreted by the secretory phase and pregnancy endometrium as well as by ovarian granulosa cells in vitro. In this study, we examined the cellular localization of IGFBP-1 in female reproductive tissues by using a purified monoclonal antibody Mab 6303 with an immunoperoxidase technique. Proliferative- and early secretory–phase endometrium as well as all extrauterine tissues except decidualized cells at the implantation site on the ovaries of ovarian pregnancies were negative for IGFBP-1. In midsecretory-phase endometrium, focal staining was first observed in the cytoplasm of glandular epithelial cells, with weaker staining in the stromal cells. In late secretory–phase endometrium, strong immunostaining was observed in predecidualized stromal cells, with weak focal staining remaining in some of the glandular epithelial cells. In early pregnancy, intense staining was detected in the cytoplasm of decidualized stromal cells of zona compacta in each sample, whereas the nondecidualized stromal cells remained unstained. Strong to medium staining was detected simultaneously in the glandular epithelial cells in 70% of the early pregnancy specimens. In term pregnancy, IGFBP-1 was localized in decidual cells of placental bed and decidua parietalis. Immunolocalization of IGFBP-1 to both endometrial epithelial and stromal cells, although only stromal cells express the gene of IGFBP-1 [14], supports the hypothesis of paracrine actions between these cells. The localization of IGFBP-1 to decidualized cells at the extrauterine implantation sites implies its association with decidual differentiation. **Endocr Pathol 2:132–138, 1991.**

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Insulinlike growth factors I and II (IGF-I, IGF-II) are polypeptides that stimulate cellular proliferation and differentiation and have insulinlike anabolic effects [3]. The biological actions of IGFs are mediated through cell membrane receptors [20]. In serum, amniotic fluid, and cell culture supernatants, IGFs are bound to binding proteins [3, 4]. Recent evidence suggests that binding proteins function as local regulators of IGF actions [10, 11, 18, 21, 25]. Three distinct IGF binding proteins (IGFBPs) have been extensively characterized in the human [2]. IGFBP-1, which has a molecular weight of 25K based on its amino acid sequence [8, 9, 13, 15], is a minor binding protein in adult serum but is found in highest concentrations in amniotic fluid [5, 8, 9, 13, 19]. This protein has been known by different names in the previous literature: BP-25 [15], IGF binding protein 1 (IBP-1) [9], placental protein 12 (PP12) [13], 34K binding protein [25], BP-28 [5], 31,000-dalton IGF binding protein [10], and pregnancy-associated endometrial α_1-globulin (α_1-PEG) [29]. IGFBP-1 has been shown to be synthesized by human secretory-phase and pregnancy endometrium [23, 24] and human granulosa cells [26]. Two forms of IGFBP-1

with similar sequences but different biological actions have been described [10]. One form inhibits [18, 21, 25] while another enhances the receptor binding of IGF-I [10, 11]. Therefore, the binding protein may either inhibit or stimulate the biological actions of IGFs. The ovarian granulosa cell has been shown to be a site of IGF-I production, reception, and action [1]. The presence of IGF-I receptors in the endometrium [18, 25] and the cyclic secretion of IGFBP-1 suggest a role for IGFs also in human endometrial function. The endometrial cell types secreting IGFBP-1 remain unclear, and the data on the cellular localization of IGFBP-1 in the endometrium and other reproductive tissues are inconsistent [27–30]. In this study, we used purified monoclonal antibodies against PP12 preparation [22] to reevaluate the cellular distribution of IGFBP-1 in female reproductive tissues in the pregnant and nonpregnant state.

Materials and Methods

Tissue Samples

A total of 118 formalin-fixed paraffin-embedded tissue samples were studied. Samples included 28 specimens of the endometrium obtained either by curettage or at hysterectomy performed for benign uterine disease (fibroids, endometriosis, cervical dysplasia) at various phases of the menstrual cycle. In all cases, adjacent hematoxylin and eosin–stained paraffin sections with good morphological details were evaluated to determine the cellular composition and for histological dating of the endometrial specimens [17]. Five tissues represented the proliferative phase (days 2–14), and 6, 10, and 7 samples represented the early (days 15–18), middle (days 19–23), and late (days 24–28) secretory phases, respectively, as judged by histological dating. No endocrinological data were available. Decidual and placental tissues were collected from early intrauterine (n = 20) and term (n = 6) pregnancies. Early pregnancy samples came from patients having legal or spontaneous abortions. Specimens of the ovary (n = 5) from ovarian pregnancies were also stained. Other tissues from the female reproductive tract included specimens from uterine cervix, myometrium, myoma, ovary, and fallopian tube from nonpregnant women. The major organs from adults and fetus, including liver, kidney, spleen, brain, heart, and gastrointestinal tract, were stained to determine the tissue specificity of the monoclonal antibodies.

Antibodies

The mouse monoclonal antibodies (Mabs) against human IGFBP-1 were kindly provided by Medix Biochemica, Kauniainen, Finland. The purification and characterization of the antibodies, which were prepared against purified PP12 (Behringwerke, Marburg, Lahn, West Germany) [7], have been described elsewhere [22]. The purified antibodies Mabs 6301, 6302, 6303, and 6305 were tested in dilutions 1 : 100 to 1 : 1,000. The staining intensity was strongest and most consistent with Mab 6303 and, therefore, this antibody was selected for the final study and was used in a 1 : 500 dilution. The interaction of Mab 6303 with IGFBP-1 is not affected by the presence of IGFs [22].

Immunohistochemical Staining

The avidin-biotin immunoperoxidase staining was carried out using Vectastain ABC Kit (Vector Laboratories, Inc., Burlingame, CA). One slide of each tissue specimen was stained with hematoxylin and eosin for histological examination. The staining procedure was carried out at room temperature in a humid chamber. All washes were done using 0.01M phosphate-buffered saline (PBS), pH 7.4. Tissue sections were deparaffinized in xylene and rehydrated by sequential incubation in ethanol. Endogenous peroxidase activity was quenched by incubation with 0.3% hydrogen peroxide. Following careful washing, the sections were incubated with the blocking serum for 20 minutes. The monoclonal antibody against IGFBP-1 diluted in PBS containing 0.5% Tween was applied for 1 hour, followed by biotinylated antimouse antibody for 30 minutes and then by Vectastain ABC reagent for 60 minutes. After washing, the sites of antibody binding were demonstrated by developing the peroxidase reaction with 3,3-diaminobenzidine (10 mg/ml) in TRIS buffer, pH 7.4, or with 3-amino-9-ethyl-carbazole in acetate buffer, pH 5, both containing 0.03% H_2O_2. The sections were counterstained with Harris

hematoxylin. Control sections were stained by replacing the first antibody with an anti-factor VIII monoclonal antibody of the same immunoglobulin class as IGFBP-1 antibody.

Results

No immunostaining was detected in any of the samples representing proliferative- or early secretory-phase endometrium (Fig. 1A, B). In the midsecretory phase, IGFBP-1 was observed in the cytoplasm of the glandular epithelial cells in 2 of 10 tissue samples (Fig. 1C) and in the stromal cells in 2 of the 10 samples, 1 additional sample showing positive staining in both cell types. The staining was focal in all samples, and its intensity varied from weak to medium in different samples. In the late secretory phase, IGFBP-1 immunoreactivity was localized solely in the cytoplasm of glandular epithelial cells in 1 of 7 samples and, in 5 of 7 samples, strong staining was found in the predecidualized stromal cells of zona compacta (Fig. 1D). In one of these samples, weak focal staining was detected in the glandular epithelial cells simultaneously. The frequency of the positively stained predecidual cells, as well as their staining intensity, increased toward the end of the nonconceptional cycle.

In early pregnancy endometrium, strong cytoplasmic staining was observed in decidualized stromal cells of zona compacta in all 20 samples studied, whereas the undifferentiated stromal cells remained unstained (Fig. 1E). In 14 samples (70%), IGFBP-1 was present in the cytoplasm of glandular epithelial cells as well. Staining was found in epithelial cells of both secretory and hypersecretory glands. In 5 cases, the staining intensity was equal in decidualized stromal cells and epithelial cells. In 6 cases, it was stronger in decidualized stromal cells and, in 3 cases, staining was stronger in the glandular epithelium. Medium to strong staining was also found in glandular secretions of some glands. There was no difference in the staining pattern between the samples from legal and spontaneous abortions.

In late pregnancy, when glandular epithelium has regressed, the immunoreactivity was found solely in decidual cells of decidua parietalis and basalis. Cytotrophoblasts and syncytiotrophoblasts in chorionic villi were negative in both early and term pregnancy.

In tissues other than endometrium, medium to strong immunostaining was detected in decidualized cells at the implantation site of ovarian pregnancies in the ovary (Fig. 1F). Myometrium, uterine cervix, corpus luteum, and the epithelium of the fallopian tube in nonpregnant women were all negative when stained with the same antibody dilution as the endometrial samples. All extragenital adult tissues as well as all fetal tissues were negative. No immunostaining was detected when an inappropriate monoclonal antibody was substituted for Mab 6303.

Discussion

In this study, we used purified monoclonal antibody Mab 6303 with an avidin-biotin immunoperoxidase method to examine the cellular localization and distribution of IGFBP-1 in female reproductive tissues. The previous localization studies on this protein have used polyclonal PP12 antiserum [7, 27, 28] and monoclonal antibodies against α_1-PEG [29, 30]. The results of those studies are inconsistent and, therefore, the cellular distribution of IGFBP-1 in the endometrium has remained unclear. Polyclonal PP12 antiserum also reacted with trophoblastic cells [7, 27].

In keeping with the previous observations, no immunoreactive IGFBP-1 was found in proliferative- or early secretory-phase endometrium [28, 29]. In the midsecretory phase, half of the samples were positive for IGFBP-1 and, in the late secretory phase, the positivity rate was 80%. The staining pattern of IGFBP-1 in the middle and late secretory phases was not consistent and, furthermore, the cellular distribution of positive staining in predecidualized endometrium (zona compacta) of the nonconceptional cycle appeared to be different from that of early pregnancy endometrium. In midsecretory phase, the staining was more intense in glandular epithelial than in stromal cells but, with advancing stromal cell differentiation, the immunoreactivity switched from the glandular epithelial cells to predecidualized stromal cells, which showed the strongest

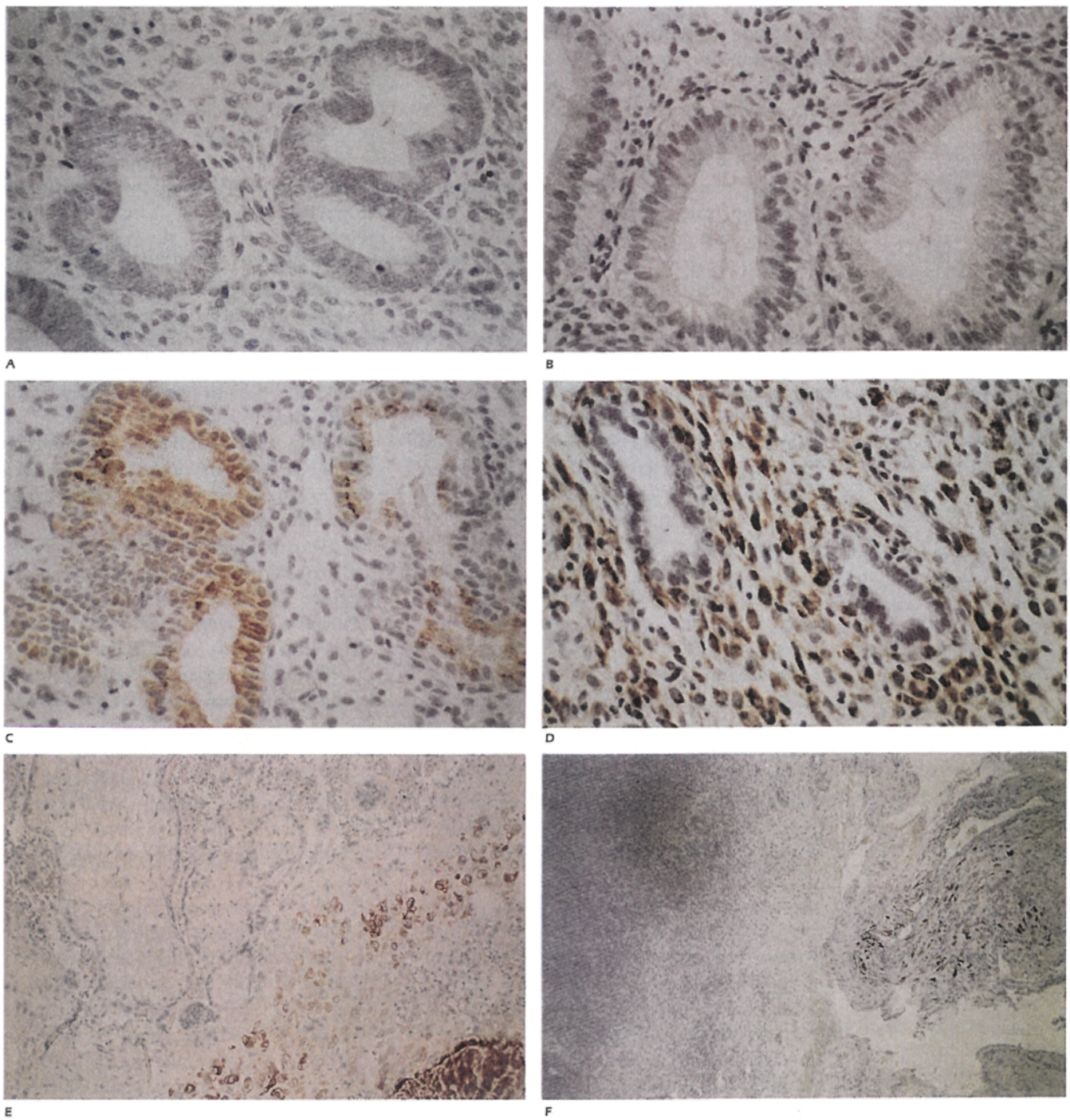

Figure 1. Immunoperoxidase localization of insulinlike growth factor binding protein-1 (IGFBP-1) with monoclonal antibody Mab 6303 in the human cyclic and gestational endometrium and ovary in ovarian pregnancy. (A) Proliferative endometrium. Endometrial glands and stroma are negative for IGFBP-1. (B) Early secretory endometrium with glands showing prominent basal vacuolation. Glands and stroma are negative for IGFBP-1. (C) Midsecretory endometrium (day 22), in which glands are stained for IGFBP-1 (brown), while the stroma is unstained. (D) Late secretory endometrium (day 27). Predecidualized stromal cells are strongly stained for IGFBP-1. Endometrial glands are unstained. (E) Gestational endometrium. Both hypersecretory glands and decidualized stromal cells are strongly immunoreactive for IGFBP-1; trophoblastic villi are unstained. (F) Ovarian tissue with decidualized cells staining strongly for IGFBP-1. (Hematoxylin counterstain; original magnification A–E ×160, F ×40.)

staining intensity in premenstrual endometrium. The switch of immunostaining of IGFBP-1 from glandular to stromal cells with advancing endometrial differentiation, as shown in this study, is of interest since endometrial epithelial cells differentiate during the first postovulatory week and stromal cells during the second postovulatory week [17]. That the staining pattern was not uniform in all parts of the specimens is not surprising, since it is well established that even the histological changes in the endometrium are never uniform. Some cells or regions reveal greater differentiation than cells in adjacent parts, although they are exposed to similar hormonal milieu. It is of interest to note that part of the endometrial specimens, although in the middle or late secretory phase by histological criteria, were negative for IGFBP-1. Whether this reflects a biochemical deficiency with possible other consequences in endometrial function remains to be evaluated in a larger series of samples.

The synthesis of IGFBP-1 by the endometrium depends on progesterone stimulation, being thus greatly enhanced during pregnancy [13, 23, 24]. In early pregnancy endometrium, medium to strong staining was detected in the cytoplasm of decidualized stromal cells in every sample and, in 70% of cases, positive staining for IGFBP-1 was found in glandular epithelial cells as well. Interestingly, the nondecidualized stromal cells remained unstained even in pregnant endometrium. The variable staining patterns of IGFBP-1 in the endometrium in the nonconceptional cycle and in early pregnancy may reflect differences in epithelial and stromal cell response to hormonal stimuli in these different circumstances. In the late luteal phase of the nonconceptional cycle, the corpus luteum is regressing, whereas after conception it is thriving and its progesterone production is increasing. The difference in the staining pattern of IGFBP-1 may reflect differences in either production or action of this protein by the cells in question. In agreement with the results obtained with monoclonal α_1-PEG antibodies [30], immunostaining was also detected in glandular secretions and in extracellular matrix in some samples. This is not surprising considering the secretory nature of IGFBP-1. The almost complete absence of epithelial cells in term pregnancy and the localization of staining in decidual cells of placental bed and decidua parietalis suggest that decidualized stromal cells are the major source of IGFBP-1 in term pregnancy decidua. Localization of IGFBP-1 to decidualized cells in the ovary in ectopic pregnancies suggests that IGFBP-1 is involved also in extrauterine implantation.

The lack of IGFBP-1 immunostaining in trophoblastic cells is in contrast with the results obtained with the polyclonal antiserum against PP12 [7, 27] but consistent with the data from a number of recent studies that have been unable to demonstrate the presence of this protein or its mRNA in placental tissue or trophoblastic cells [9, 13, 21]. Why PP12 was localized to trophoblastic cells by polyclonal antiserum remains unclear [7, 27]. The abundance of IGFBP-1 in the decidual cells accounts for the original detection of this protein in extracts of term human placenta and membranes [7].

Human hepatoma HEPG2 cell line [15] and explants of fetal liver have been shown to produce IGFBP-1 [16]. The specimens of fetal and adult liver, however, were negative when stained with Mab 6303 using the same or even tenfold higher antibody dilution than for the endometrial samples. Since Mab 6303 immunoprecipitates HEPG2-conditioned medium, an immediate secretion of IGFBP-1 out of the liver cells or a difference in the intracellular conformation of IGFBP-1 in liver cells and in endometrial cells might account for the inability of Mab 6303 to detect IGFBP-1 in the liver specimens. Waites and colleagues [30] were also unable to stain fetal tissues with monoclonal antibodies against α_1-PEG. This is in contrast to the findings of Hill and coworkers [12], who used polyclonal antiserum against pure IGFBP-1 derived from amniotic fluid. Their data showed positive staining for IGFBP-1 in every fetal tissue except brain, spleen, and thyroid. Interestingly, the antiserum used in the latter study was raised against a form of IGFBP-1 that adheres to cell surface and enhances the receptor-binding and biological action of IGF-I [10, 11], whereas PP12 and α_1-PEG have opposite effects [6, 18, 21, 25]. This difference in antigen forms may account for the discrepancy in results obtained with different antibodies in fetal tissues.

Recently, human granulosa cells were shown to produce IGFBP-1 [26]. In this study, however, ovarian tissues obtained from nonpregnant women remained negative when stained with Mab 6303, while decidualized cells in the ovary of ovarian pregnancies were positive. Two possible explanations for this observation are, first, the number of granulosa cells producing IGFBP-1 may be too small to be detected in all ovarian samples and, second, the gene expression of IGFBP-1 may be limited to only a precise state of granulosa cell differentiation, similar to what has been speculated to occur in decidual cells [14].

In conclusion, our data demonstrate that IGFBP-1 is associated with both glandular epithelial and decidualized stromal cells in human endometrium, the cellular distribution changing in the cyclic and gestational endometrium. Immunolocalization of IGFBP-1 to both epithelial and stromal cells, and in situ hybridization to stromal cells only [14], supports the concept of paracrine actions between these cell types. Alternatively, the immunoreactivity in the epithelial cells could represent a different BP species with an epitope in common with IGFBP-1. This is, however, unlikely since other tissues known to contain different IGFBPs remained negative when stained with Mab 6303. In addition, we show for the first time that IGFBP-1 is present in decidualized cells at the implantation site of ectopic pregnancy. It will be of interest to examine whether the alteration in the cellular distribution of IGFBP-1 in the endometrium may reflect different functions for this protein at different stages of endometrial growth and development and decidual transformation. The negative immunostaining of other than endometrial and decidual cells suggests that the monoclonal antibodies used in this study are directed especially to endometrial epitopes of IGFBP-1 and thus provide a new valuable tool for investigating the physiological functions of this protein in female reproduction.

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