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Embryo-maternal dialogue in the primate: regulation of insulin-like growth factor binding protein (IGFBP-1)

Diálogo materno-embrionario en primates: regulación de la proteína ligante del factor de crecimiento de tipo insulínico

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INTRODUCTION

There has been increasing appreciation in recent years for the importance of fetal and maternal tissue interaction in pregnancy. Numerous interactions are associated with this relationship, the most important of which is the achievement of implantation and the maintenance of pregnancy. Embryo implantation is the natural culmination of the menstrual cycle, but successful nidation requires the precise preparation of both the blastocyst and endometrium. A remarkable synchrony is achieved by continuous maternal/conceptus interaction even prior to trophoblastic invasion. The internal lining of the uterus is a specialized interface where a complex combination of anatomic, biochemical, endocrinologic and immunologic events occur to ensure successful embryonic development. It is apparent therefore, that the biological requirements of the early mammalian conceptus must be met by uterine and oviductal secretions, since they constitute the primary environmental contact between the developing embryo and its mother prior to implantation.

Although significant progress has been made in farm animals regarding conceptus secretory products and the maternal recognition of pregnancy (1), the maternal-fetal interactions in the primate are poorly understood. Moral and ethical limitations on human research associated with the lack of an appropriate non-human primate model have been the major limitations in these types of studies. Our studies (2-5) have established that the baboon would be an excellent non-human primate model for attempting to understand interactions between mother and fetus during early embryonic development. Although morphological differences do exist in the implantation processes of the human and baboon embryo (6, 7), the fact that the endometrium of both species produce similar molecules during pregnancy (4, 5, 8) coupled with the synthesis of common conceptus hormones, provides for biological markers that can be utilized for studying conceptus-maternal interactions in the primate.

Preimplantation embryos from several species secrete substances that assist in implantation. Pig blastocysts begin to synthesize and secrete estrogen at the onset of rapid trophoblast elongation (9-11) which in turn results in an increase of calcium, acid phosphatase and protein in uterine flushings (12). Blastocyst estrogen appears to be the major luteotrophic signal in the pig (13, 14) and the regulator of endometrial protein release at the time of blastocyst attachment (15). Ovine trophoblast protein 1 (oTP-1) is the best-characterized of the conceptus derived proteins. oTP-1 is reported to suppress endometrial prostaglandin $F_{2\alpha}$ release (16) and modify endometrial luminal secretions (17-19). It is the predominant secretory product during the time of maternal recognition of pregnancy in the sheep.

Bovine conceptus culture media has also been shown to selectively induce the

synthesis of two bovine endometrial proteins in culture (20) and oTP-1 is also able to enhance the synthesis of some proteins in sheep endometrial epithelial cell cultures (19). The asynchronous transfer of day 6 sheep embryos to day 4 recipients results in the acceleration of the endometrial secretory response to one similar to the synchronous transfer control (21). The observed effects in the sheep and cow may be due to the action of interferon since the ovine and bovine conceptus proteins, oTP-1 and bTP-1, are structurally related to the alpha interferons, and the oTP-1 and the alpha interferons have been shown to alter endometrial protein synthesis (19, 22). The first well defined secretion of the primate embryo is chorionic gonadotropin. The physiological function of chorionic gonadotropin is to take over the trophic support of the corpus luteum and is therefore essential for embryonic survival. However, unlike the conceptus secretory products of the domestic species, it is unclear whether chorionic gonadotrophin or other primate conceptus secretory products have a local function at the site of implantation.

A significant body of literature exists regarding proteins secreted by the endometrium in many species (23, 24). Many of these proteins have been shown to be different from those present in plasma and the synthetic pattern of many of these proteins are correlated with the plasma levels of the ovarian steroids. However, very few of these proteins have been purified or characterized, and it has been even more difficult to demonstrate whether these secretory macromolecules are essential in the implantation process. We have shown that the baboon endometrium during explant culture synthesizes and releases at least 15 to 17 polypeptides (2). These polypeptides can be divided into two groups, group I proteins are those that are present throughout the menstrual cycle and only show minor cyclic variation in synthesis, and group II proteins are those whose secretion appears to be hormonally modulated (2, 3). In the first group, proteins with molecular weights of 66,000; 46,000 and 37,000 are electrophoretically similar to previously described human endometrial proteins (25). The second group includes a M_r 33,000 and a M_r 40,000 protein which also appear to be electrophoretically similar to ones synthesized by the human endometrium (2, 3). Two additional proteins, that may have functional significance, have been identified as the major secretory products of the human endometrium (25). Endometrial protein 15, also known as α_2 pregnancy-associated endometrial globulin (α_2-PEG), is the major secretory product during the late luteal phase and early pregnancy (25). This protein is identical to placental protein 14 and has sequence homology with β-lactoglobulin and serum retinol binding proteins. It has been suggested that α_2-PEG may be involved in retinol transport during implantation and early embryo development (25). Interestingly, although α_2-PEG constitutes the major endometrial secretory product in the human, it is absent in the baboon (3, 25). In contrast, α_1-PEG, which is IGFBP-1 and becomes the major secretory protein by the fifteenth week of gestation in the human (23, 25), is also produced by the baboon (3-5). This protein is biochemically identical in every respect in both species (15). However, its site of synthesis during the menstrual cycle differs markedly when a specific monoclonal antibody is utilized (4). The site of production during the late luteal stage in the baboon is the epithelium of the deep glands (4), while in the human synthesis is confined to the predecidualized cells of the stroma (26). During pregnancy, however, the site of synthesis in both species is the hypertrophied stromal cells of the decidua (5).

POTENTIAL FUNCTION OF IGFBP-1 IN THE BABOON

The somatomedins, IGF-I and IGF-II are thought to act via endocrine, paracrine and/or autocrine mechanisms (27, 28) and their binding proteins are thought to regulate their action (29). The close association between the developing trophoblast and decidualized endometrium together with evidence for IGF production

by placental tissues (30), suggest that decidual IGFBP-1 in the baboon and human may regulate feto-placental growth. The IGF's are a family of olygopeptides related to insulin which stimulate growth and differentiation in a number of tissues during fetal development and post-natally (31, 32). These peptides (IGF I & II) bind to specific receptors on the cell surface and are usually found complexed to soluble binding proteins (33). These IGFBP's bind IGF's with comparable affinity to that of their receptors and are able to modulate IGF action (34, 35).

In the pregnant baboon, IGFBP-1 is localized to the decidual tissue (5) during the third trimester. However, in the non-pregnant baboon, immunocytochemical staining with the same antibody, localized IGFBP-1 to the endometrial epithelium of deep glands during the luteal stage of the cycle (4). Since it is not certain what factors regulate IGFBP-1 synthesis and secretion in the baboon endometrium, nor what may be regulating the switch in the cell-type which secretes IGFBP-1 during pregnancy, we have attempted to characterize the secretory profile of this protein in the pregnant baboon between days 18 and 34 post-ovulation. The implantation site was identified by ultrasound and following hysterectomy the endometrium was separated into 3 regions: region 1 directly below the implantation site, region 2 adjacent to the implantation site, and region 3 opposite the implantation site. Immunocytochemical studies using the monoclonal antibody to IGFBP-1 demonstrated intense glandular epithelial staining in all regions on days 18, 25, and 31 postovulation. Stromal staining for IGFBP-1 was not apparent on day 18, but intense staining was evident in the stromal cells intimately in contact with the trophoblastic tissue on day 25. The deeper stromal cells did not express IGFBP-1 nor did the stromal cells in regions 2 or 3. By day 31 IGFBP-1 expression was not limited to the endometrial-trophoblastic junction, but extended to the deeper stromal cells and included the perivascular regions. Ligand blot analysis of TCM using ^{125}I-IGF-1 showed that the functionalis appeared to express the majority of IGFBP-1 in all regions on days 18, 25 and 31, but by day 31 the basalis also synthesized the protein, thus confirming the immunocytochemical data (Tarantino, Verhage and Fazleabas, unpublished data).

The biological function of IGFBP's have yet to be convincingly demonstrated. They may perform a storage or transport function, or they may be responsible for modulating the actions of IGF (34, 36-39). It has been suggested that IGFBP-1 synthesized by the human endometrium may play a role in either regulating trophoblast growth or permitting proliferating stromal fibroblasts to respond to endogenous IGF (23, 36). Recent evidence suggests that the IGF-I/IGFBP complex is a better mitogen than free·IGF-1 (40). Following the initial wave of rapid trophoblast invasion, implantation appears to be more stringently controlled in the baboon compared to the human. Thus, one might suggest that IGF BP-1 may play a role in regulating trophoblast penetration. Perhaps, the IGFBP-1 complexed with IGF and localized in the glandular epithelium acts as a mitogen on conceptus tissues and facilitates trophoblast penetration and contact with the maternal vasculature. Following implantation, the switch in the site of synthesis in the baboon, and the continued rise in IGFBP-1 synthesis by the decidual cells in the human, may then be responsible for stromal proliferation and control of trophoblast invasiveness.

Production of IGFBP-1 by primate decidual tissue must reflect some unique requirement in pregnancy in species exhibiting hemochorial placentation (41). The function of IGFBP-1 may be associated with the autocrine and/or paracrine regulation of trophoblast growth and regulation by modulating the bioavailability of IGF's. The autocrine function of IGFBP-1 could be to regulate IFG action on endometrial/decidual cells, whether IGF's are produced endogenously or exogenously. The endometrium undergoes dramatic growth and differentiation during the first trimester, and if IGF's are required for these processes, the local production of IGFBP-1 in its stimulatory form could

provide a mechanism to enhance only locally, the growth promoting effects of IGF.

Our preliminary studies during early pregnancy suggest that the conceptus may regulate the site and cell specific expression of IGFBP-1 in the baboon endometrium. The regulation of the IGF/IGFBP-1 complex by trophoblast tissue in the glandular epithelium and hypertrophied stromal cells at the site of implantation may be of critical importance to promote both cellular proliferation during the initial stages of attachment and control the invasive potential of the trophoblast cells following implantation. A question to be addressed is whether this reflects the mode of implantation in species where the process is superficial as in the baboon or invasive as in the human (42, 43). We are currently attempting to address these questions with special reference to the expression and distribution of IGF's, their receptors and BP's in the various uterine and conceptus cell populations during implantation and early pregnancy.

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