Dialogue between Blastocyst hCG and Endometrial LH/hCG Receptor: Which Role in Implantation?

S. Perrier d'Hauterive[a, b]   S. Berndt[b]   M. Tsampalas[a]   C. Charlet-Renard[a]
M. Dubois[b]   C. Bourgain[c]   A. Hazout[d]   J.-M. Foidart[b]   V. Geenen[a]

[a]Department of Medicine, Center of Immunology, Institute of Pathology CHU-B23, University of Liege, Liege-Sart Tilman, [b]Department of Gynecology and Obstetrics, CHR-Citadelle, University of Liege, Liege, [c]Department of Pathology, Free University of Brussels University Hospital (AZ-VUB), Brussels, Belgium; [d]Center of Reproductive Medicine, Bichât Hospital, Paris, France

Abstract

The specific interaction of blastocyst-derived human chorionic gonadotropin (hCG) and endometrial LH/hCG-R constitutes a fundamental component of the molecular dialogue at the materno-fetal interface. From our observations and studies from other groups, hCG was indeed shown to play a significant role in implantation and tolerance of the embryo, decidual differentiation and remodeling, as well as in placentation. The profile pattern of *LH/hCG-R* expression by endometrial epithelium correlates with the theoretical timing of the implantation window. Studies are currently being conducted in assisted medical procreation and in an animal model of implantation to establish the index of *LH/hCG-R* expression as a new biomarker of uterine receptivity for embryo implantation.

Copyright © 2007 S. Karger AG, Basel

Introduction

Implantation of the embryo into the maternal endometrium represents a crucial step in the reproductive process in several species. It arrives from a cascade of finely tuned events leading to apposition, adhesion and then invasion of blastocyst in the uterine wall. Successful pregnancy requires a synchronized dialogue between receptive endometrium and functionally normal embryo. Though sexual steroids control the process, a network of cytokines are the private paracrine mediators of the dialogue at the maternal-embryonic interface [1, 2]. The cross-talk between these two protagonists and their reciprocal effects constitute an unsolved problem of reproductive medicine. Whereas an embryo could be implanted in any human tissue, the endometrium is the only one in which implantation cannot occur except during one limited period called the implantation window [3]. During this stage, it offers a high receptivity for the blastocyst. To date, there is no molecular definition of this implantation window. The only marker is the implantation itself, associated with the appearance of human chorionic gonadotropin (hCG) in the maternal bloodstream.

hCG is the prime mediator by which the embryo announces its presence to the maternal organism since it produces it even before its implantation.

In our research program, we were particularly interested in the role of this embryo-specific signal in the implantation process and in the impact of its receptor (the LH/hCG receptor) as a possible marker of the implantation window.

Uterine Receptivity

Implantation of the embryo in the uterus is a restricted phenomenon which is only possible during a short period called the implantation window [4]. The study of the human implantation window is a difficult challenge burdened with a series of redundant factors coexisting at the materno-fetal interface. Several factors such as pinopods, integrins, mucins, heparin-binding epidermal growth factor or steroid receptors have been evidenced and extensively studied, but no particular one can be highlighted. For a prognostic and clinical value in assisted medical procreation (AMP), a good receptivity marker must be evidenced with a simple and not traumatic method in the cycle during which an embryo could possibly be implanted. Moreover, uterine receptivity has to be attested cycle after cycle. Actually, the only specific but retroactive proof of uterine receptivity is the implantation itself and the production of hCG.

Human Chorionic Gonadotropin

hCG belongs to the family of glycoprotein hormones, such as LH, FSH and TSH. These hormones are composed of two subunits linked in a non-covalent way. The α-subunit is common to the whole members of the family and is coded on chromosome 6. The β-subunit, different for each hormone, is coded by distinct genes on chromosome 19 (LH, hCG and TSH) or chromosome 11 (FSH). The β-hCG is the biggest β-subunit because of a larger glycosylated part. This important glycosylation confers to hCG a higher stability and makes its secretion faster. The β-subunits of LH and hCG have a 96% identity that allows these hormones to share the same receptor. However, hCG binds to this receptor with an affinity 4.5-fold higher compared to LH. hCG is principally produced by the trophoblast (and particularly the syncytium), but also by some malignant tumors.

LH/hCG Receptor

LH/hCG-R is a member of the G-protein-coupled receptors and is coded on chromosome 2. This unique gene is composed of 10 introns and 11 exons, and spans approximately 80 kb. Its cDNA codes a glycoprotein of 675 amino acids. This receptor associates two functional units: a large extracellular part which serves the recognition and the specific binding of hCG (or LH) coupled to 7 transmembrane domains and an intracellular segment bound to G protein. This segment allows the signal transduction generated by the hormonal binding to the extracellular domain [5, 6]. This G-protein-coupled receptor mainly activates the cAMP/PKA pathway [7]. hCG and LH activities are mediated by the same receptor (hCG/LH-R). The expression of this receptor was previously thought to be restricted to gonadal tissue. However, its presence has been evidenced in many other tissues throughout the reproductive organs [8].

hCG-LH/hCG-R System and Implantation

At the Center of Immunology of Liege University, we are studying the impact of hCG on implantation as well as the expression of LH/hCG-R by the human endometrium. First of all, on the mRNA level by RT-PCR carried out with specific primers, we evidenced *LH/hCG-R* transcripts in the whole endometrial tissue and in stromal and epithelial cells isolated from endometrial biopsies of fertile women [9]. Through real-time PCR, we quantified *LH/hCG-R* expression in endometrial epithelial cells isolated from biopsies during the proliferative phase and secretory phase. Our preliminary results demonstrated an increase of expression in the mid-luteal phase, during the supposed implantation window. It should, however, be noted that the basal expression of the receptor is weak. The kinetics of expression of *LH/hCG-R* suggests that this receptor could constitute a marker of endometrial receptivity. The relation between the level of expression of *LH/hCG-R* and uterine receptivity must, however, still be elucidated by other experiments. We also sought the presence of LH/hCG-R at the protein level. The capacity of the hCG to be fixed at the endometrial epithelial cells was shown by a binding experiment using of hCG marked with fluorescein.

Endometrial LH/hCG-R is functional since hCG (1–50 IU/ml) was able to stimulate in vitro the production of leukemia inhibitory factor (LIF), a cytokine primordial in murine implantation [10]. hCG also inhibited the production of an immunoactivatory cytokine, interleukin-6 (IL-6) [9]. These effects were inhibited by an anti-hCG antibody, and were not mediated by local IL-1β. However, only the dimeric form of the hCG fulfills these biological functions since the α- and β-subunits sepa-

rately did not induce any modulation of the endometrial cytokine production. Finally, hCG also stimulates the endometrial production of a potent pro-angiogenic factor, the vascular endothelium epidermal growth factor (VEGF).

Our results strongly suggest that the embryo actively participates in the first steps of implantation. Indeed, through hCG production, the blastocyst stimulates the endometrial production of pro-implantatory LIF and pro-angiogenic VEGF, while it reduces the pro-inflammatory IL-6 following a specific interaction with endometrial LH/hCG-R [9–11] (fig. 1).

hCG-LH/hCG-R System and Initiation of Pregnancy

hCG is one of the most specific and precocious molecules produced by the embryo. Indeed, ARNm of the hCG is transcribed as soon as the two-cell stage [12]. At the blastocyst stage, transcripts of hCG are detected in the trophoblast and hCG production by the blastocyst begins before its implantation. Significant rates of hCG can be measured in maternal blood 10 days after ovulation. The peak of hCG production by the placenta is reached between the 10th and 11th week of gestation, then the synthesis declines as of the 12th week, remaining at a low rate throughout pregnancy. From current studies, hCG positively influences the implantation not only by its luteotrophic role, but also via a local action at the maternofetal interface, through specific interaction with LH/hCG-R. It is increasingly clear that hCG intervenes in the regulation of endometrial differentiation and in the implantation process with an impact at the different steps of the implantation [13–15].

Pro-Implantatory Role of hCG

Administration of hCG by microdialysis in human uterus causes important paracrine effects on decidualization, suggesting that the embryo increases the duration of the implantation window, on tissue remodeling (increase of MMP-9), on implantation (LIF, M-CSF), and on angiogenesis (VEGF) [16]. The treatment of endometrial epithelial and stromal cells with hCG increases expression of *COX-2*, via the AMPc/protein kinase A signaling system [17]. The COX-2 enzyme, which catalyzes formation of prostaglandins (PGE_2 in particular which supports the differentiation of the stroma cells in decidua), is absolutely essential for implantation. Targeted inactivation of COX-2 in the mouse leads to severe anomalies of reproduction, e.g. failure of implantation [18].
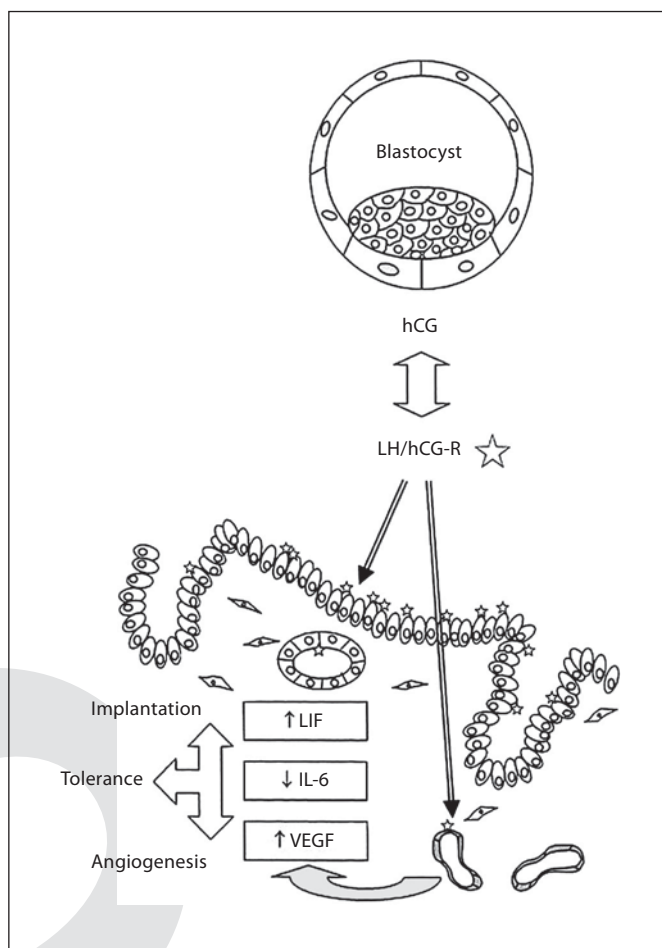

Fig. 1. Impact of the embryo on the implantation process, maternal immune tolerance and angiogenesis: hCG, by interacting through its endometrial receptor, LH/hCG-R, increases epithelial production of LIF and VEGF, decreases epithelial production of IL-6 and increases vascular endothelium production of VEGF.

hCG and Angiogenesis

The presence of LH/hCG-R on endothelial cells of the uterine vessels has already been described [19]. Toth et al. [20] showed that the in vivo administration of hCG reduces vascular resistance in the human uterus and reduced in vitro the vasoconstrictor eicosanoids of the vascular wall. These results initiated a study in a population of patients presenting signs of miscarriage. These patients were treated with magnesium or progesterone and/or hCG. The results showed that the number of patients who reached the second trimester of pregnancy was higher when hCG was included with the therapeutic protocol, in parallel with a reduction of vascular resis-

tance. Moreover, the number of premature births or intrauterine growth retardation was weaker when hCG had been managed in the first trimester. Zygmunt et al. [21] have recently proposed hCG as new angiogenic factor. Using a system of in vitro angiogenesis in 3D, they showed that hCG is a factor promoting angiogenesis by supporting the migration and the formation of outlines of capillaries by the uterine endothelial cells. In order to further understand the implication of the hCG in the implantation process, we studied the impact of hCG using in vitro treatment on the production of VEGF by endometrial epithelial cells and on uterine angiogenesis. Our results suggest that endometrial epithelium is able to answer the signal specifically sent by an embryo in the process of affixing, by the production of VEGF which then stimulates angiogenesis on the level of the subjacent vessels. In addition, the invading embryo continues to produce hCG which is able to stimulate the angiogenesis directly [22].

Role in the Maternal Tolerance of the Fetal Allograft

In parallel with its direct action on endometrium (epithelium and stroma), hCG also contributes to maternal tolerance of the embryo. This function, demonstrating the intimate interrelationship between the immune and endocrine systems, has been described in several studies. Kayisli et al. [23] suggested that hCG could be a key placental factor for the development of the local immune tolerance through the cellular system of programmed death Fas/Fas ligand. In a more general way, Khan et al. [24] showed that the administration of hCG to non-obese diabetic (NOD) mice before the beginning of the clinical symptoms reduced the increase in glycemia, reversed establishment of insulitis, and inhibited the development of autoimmune diabetes.

Perspectives

Among the important number of factors that intervene in establishment of uterine receptivity, none has definitively proven its predictive and prognostic value. For a marker of uterine receptivity to be used in AMP, it should be evaluated in a simple, fast and sure way. If it is required apart from a cycle where an embryo will be transferred, it must be able to attest stable receptivity of the way from one cycle to another in the same patient and to be able to attest receptivity of a following cycle. If the markers of receptivity were evaluated at the time of embryo transfer, we could avoid the variability of the endometrium from one cycle to another, but this may raise some ethical problems, although various publications in the literature confirm the harmlessness of such biopsies.

From the expression profile during the menstrual cycle and its nature as the specific receptor for a blastocyst signal, we wish to propose LH/hCG-R as a new marker of the implantation window. In collaboration with A. Hazout (Bichât Hospital, Paris), we are investigating the index of LH/hCG-R endometrial expression as a new marker of the implantation window in a population of unfertile women during AMP treatment. These studies also include immunohistochemical analysis of other markers of the implantation window such as the integrin αvβ3, LIF and IL-10, as well as careful dating of the biopsies.

References

1 Fazleabas AT, Kim JJ, Strakova Z: Implantation: embryonic signals and the modulation of the uterine environment – a review. Placenta 2004;25(suppl):S26–S31.

2 Minas V, Loutradis D, Makrigiannakis A: Factors controlling blastocyst implantation. Reprod Biomed Online 2005;10:205–216.

3 Perrier d'Hauterive S, Charlet-Renard C, Goffin F, Foidart J-M, Geenen V: La fenêtre implantatoire. J Gynecol Obstet Biol Reprod 2002;31:440–455.

4 Perrier d'Hauterive S, Geenen V: Comprendre l'implantation en 2004. Genesis 2004; 101:15–20.

5 Dufau M: The luteinizing hormone receptor. Annu Rev Physiol 1998;60:461–496.

6 Ascoli M, Segaloff D: On the structure of the luteinizing hormone/chorionic gonadotropin receptor. Endocr Rev 1989;10:27–44.

7 Srisuparp S, Strakova Z, Brudney A Mukherjee S, Reierstad S, Hunziker-Dunn M, Fazleabas A: Signal transduction pathways activated by chorionic gonadotropin in the primate endometrial epithelial cells. Biol Reprod 2003;68:457–464.

8 Licht P, von Wolff M, Berkholz A, Wildt L: Evidence for cycle-dependent expression of full-length human chorionic gonadotropin/luteinizing hormone receptor mRNA in human endometrium and decidua. Fertil Steril 2003;79(suppl 1):718–723.

9 Perrier d'Hauterive S, Charlet-Renard C, Berndt S, Dubois M, Munaut C, Goffin F, Hagelstein M-T, Noël A, Hazout A, Foidart J-M, Geenen V: Human chorionic gonadotropin and growth factors at the embryonic-endometrial interface control leukemia inhibitory factor and interleukin-6 secretion by human endometrial epithelium. Hum Reprod 2004;19:2633–2643.

10 Stewart C, Kaspar P, Brunet L, Bhatt H, Gadi I, Kontgen F, Abbondanzo S: Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992; 359:76–79.

11 Perrier d'Hauterive S, Charlet-Renard C, Dubois M, Berndt S, Goffin F, Foidart J-M, Geenen V: Human endometrial leukemia inhibitory factor and interleukin-6: control of secretion by transforming growth factor-β-related members. Neuroimmunomodulation 2005;12:157–163.

12 Jurisicova A, Antenos M, Kapasi K, et al: Variability in the expression of trophectodermal markers β-human chorionic gonadotropin, human leukocyte antigen-G and pregnancy specific β_1-glycoprotein by the human blastocyst. Hum Reprod 1999;14: 1852–1858.

13 Cameo P, Srisuparp S, Strakova Z, Fazleabas A: Chorionic gonadotropin and uterine dialogue in the primate. Reprod Biol Endocrinol 2004;5:2–50.

14 Filicori M, Fazleabas A, Huhtaniemi I, Licht P, Rao C, Tesarik J, Zygmunt M: Novel concepts of human chorionic gonadotropin: reproductive system interactions and potential in the management of infertility. Fertil Steril 2005;84:275–284.

15 Han SW, Lei ZM, Rao CV: Treatment of human endometrial stromal cells with chorionic gonadotropin promotes their morphological and functional differentiation into decidua. Mol Cell Endocrinol 1999;147:7–16.

16 Licht P, Russu V, Lehmeyer S, Wildt L: Intrauterine microdialysis reveals cycle-dependent regulation of endometrial insulin-like growth factor binding protein-1 secretion by human chorionic gonadotropin. Fertil Steril 2002;78:252–258.

17 Zhou X, Lei Z, Rao C: Treatment of human endometrial gland epithelial cells with chorionic gonadotropin/luteinizing hormone increases the expression of the cyclooxygenase-2 gene. J Clin Endocrinol Metab 1999;84: 3364–3377.

18 Lim H, Paria B, Das S, Dinchuk J, Langenbach R, Trzaskos J, Dey S: Multiple female reproductive failures in cyclooxygenase-2-deficient mice. Cell 1997;91:197–208.

19 Toth P, Li X, Rao C, Lincoln S, Sanfilippo J, Spinnato J 2nd, Yussman M: Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. J Clin Endocrinol Metab 1994;79:307–315.

20 Toth P, Lukacs H, Gimes G, Sebestyen A, Pasztor N, Paulin F, Rao C: Clinical importance of vascular LH/hCG receptors – a review. Reprod Biol 2001;1:5–11.

21 Zygmunt M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Munstedt K, Rao C, Lang U, Preissner KT: Characterization of human chorionic gonadotropin as a novel angiogenic factor. J Clin Endocrinol Metab 2002;87: 5290–5296.

22 Berndt S, Perrier d'Hauterive S, Blacher S, Péqueux C, Lorquet S, Munaut C, Applanat M, Marie-Astrid H, Lamandé N, Corvol P, Van den Brule F, Frankenne F, Huhtaniemi I, Geenen V, Noël A, Foidart J-M: Angiogenic activity of human chorionic gonadotropin through LH receptor activation on endothelial and epithelial cells of endometrium. FASEB J 2006;20:2630–2632.

23 Kayisli UA, Selam B, Guzeloglu-Kayisli O, Demir R, Arici A: Human chorionic gonadotropin contributes to maternal immunotolerance and endometrial apoptosis by regulating Fas-Fas ligand system. J Immunol 2003;171:2305–2313.

24 Khan NA, Khan A, Savelkoul HF, Benner R: Inhibition of diabetes in NOD mice by human pregnancy factor. Hum Immunol 2001; 62:1315–1323.