Endometrial receptivity forming elements

Review article

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Summary
This paper discusses significant elements that make up the decidualization of the endometrium which renders this tissue capable of embryo implantation, i.e. makes it receptive. Subsequently, angiogenesis, penetration of the endometrium by macrophages, immune polarization and the problem or maternal immune tolerance to the embryo are discussed. A significant element for effective implantation is the site where the apical layer of the decidua communicates with the outer surfaces of the embryo. This communication is facilitated or prevented by adhesion molecules present in the endometrium. Leukemia inhibitory factor (LIF) and its receptor are particularly emphasized. Other markers mentioned in the paper are: mucin (MUC1), glycodelin A, integrins, L-selectin, interleukins (IL-1 and IL-6), E-cadherin and cyclin. Finally, a search for genetic markers of endometrial receptivity and the role of HOXA-10 and HOXA-11 genes are presented.
Key words: receptivity, endometrium, implantation, receptivity markers

INTRODUCTION

In 1976, Psychoyos proposed two terms: "endometrial receptivity" and "window of implantation" [1].

The endometrium becomes a host of an implanting embryo by preparing itself for receiving it, i.e. by becoming receptive. This entails a complex of highly coordinated biochemical and structural changes with their culmination at so-called "window of implantation," when endometrial receptivity is the highest. These changes are short-term and occur between day 7 and 9 after ovulation [2].

DECIDUAL TRANSFORMATION OF THE ENDOMETRIUM

Endometrial decidualization encompasses specific angiogenesis. The spiral arterioles are lengthened, and endothelial and smooth muscle cells proliferate. The vascular changes are accompanied by increased presence of T and B cells, macrophages and NK (natural killer) cells in the uterine wall. Macrophages, dendritic cells and B lymphocytes of the fetus play a crucial role in the immune response and antigen presentation to T cells. The cytological changes and angio-

genesis in the endometrium are stimulated by progesterone.

The presence of NK cells in the endometrium (decidua) is its important cytological feature. Their population is considerable. It is estimated that they account for approximately 70% of leukocytes in the decidua whereas this percentage in blood is only 15% [3]. It is believed that decidual NK cells play a crucial role in the process of endometrial transformation and are phenotypically and functionally different from NK cells found in blood. These cells do not show cytolytic activity and produce integrins that enable their migration and invasion into the transforming endometrium. The hypothetic role of decidual NK cells is limited to early pregnancy and consists in supporting implantation and interaction between the trophoblast and the decidua. It has been demonstrated that NK cell recruitment is controlled by progesterone and does not depend on the presence of an embryo. NK cells accumulate in the decidua at the site where it directly contacts the trophoblast. Although the actual precise function of decidual NK cells has not been explained, a range of mechanisms that can elucidate their inability to destroy semiallogenic fetal cells are being investigated. These cells do not present the expression of classical antigens: HLA-A and HLA-B, but show selective expression of HLA-C and nonclassical HLA- E_2, HLA-G and CD1d molecules, which underlies the theory that trophoblastic MHC (*major histocompatibility complex*) antigens react with NK cell receptors [4]. Using flow cytometry and RT-PCR, CD56(bright) and CD56(dim) decidual and peripheral NK cell molecules have been analyzed. The analysis revealed a three-fold increase in the expression of 278 genes in decidual cells compared with peripheral ones. Most of them encode superficial and adhesion proteins (tetraspanins CD9, CD151, CD 53, CD 63 and TSPAN-5) as well as two other proteins: galectin-1 and progesterone-associated protein 14, which are known to have immunomodulatory functions and the selective expression of which can be observed in decidual NK cells [3]. It can be therefore stated that the interactions mentioned above create a model in reproductive immunology in which elements of innate immune tolerance have been incorporated in a constructive manner to support tissues that participate in reproduction [5].

Decidualization is associated with immune polarization that consists in decreasing Th-1 and increasing Th-2 lymphocytes. These changes are promoted by progesterone and cytokines. Immune polarization is a very important process in developing immune responses since it is associated with the problem of selecting a mechanism of either humoral or cell-mediated response. It is believed that Th-2 lymphocytes protect the fetus and trophoblast as well as inhibit cytotoxicity of NK cells and activation of cytotoxic T cells. The maternal immune system adjusts itself to the profile of the developing fetal immune system which, at this stage, is dominated by the population of Th-2 cells. A major change takes place in the maternal immune system: the dominant profile of type Th-1 cell-mediated immunity is replaced by the profile of humoral immunity associated with Th-2 cell activation [6].

Maternal immune tolerance to the alien embryonic DNA and RNA, which is observed during pregnancy, is being investigated. Certain evidence enables hypotheses to be put forward that elucidate this remarkable biological phenomenon. Numerous agents that prevent maternal rejection of a fetus with alien DNA have been recognized. They include soluble and membrane-bound structures produced by both the decidua and fetus, such as: glycoproteins, HLA-class Ib products and HLA-G isoforms that occupy the materno-fetal interface and are present in the maternal blood during pregnancy. It is believed that the privilege granted to the fetus by the utero-placental system occurs when immune cells meet HLA-G (human leukocyte antigen) [7]. HLA-G is expressed on cells of certain tissues. In pregnant women, HLA-G exerts a beneficial effect by silencing immune reactions directed against the fetus. Soluble sHLA-G2 antigen plays an important role in successful embryo implantation during its transfer to the uterus [8].

A significant element for implantation is the site where the apical layer of the decidua communicates with the outer surfaces of the embryo. This communication is facilitated or prevented by adhesion molecules present in the endometrium. Various investigations have failed to indicate a single molecule that could be granted a status of "endometrial receptivity marker," but there are many agents known to be associated with the process of embryo implantation. Leukemia inhibitory factor (LIF) and its receptor have been particularly emphasized. LIF is a glycoprotein first described as a factor that induced the differentiation of murine myeloid leukemic M1 cells into macrophages, and later proposed as a marker of the embryo implantation process. The role of this glycoprotein has been shown in experiments using LIF knockout mice in which embryo implantation did not occur. In healthy women, LIF and LIF mRNA are expressed in the endometrium throughout the menstrual cycle. This expression increases strikingly in the mid-secretory phase, during the window of implantation. LIF binds to a receptor (LIFR) found on the surface of the blastocyst thus enabling it to establish contact with the endometrium. LIFR can also be found on the surface of the endometrium and its expression, like in the case of LIF, increases in the mid-secretory phase. It is believed that recombinant human LIF can increase the implantation rate in women [9].

Another molecule is MUC1 (Mucin 1, cell surface associated) – a protein anchored in the apical surface of various epithelial cells. MUC1 belongs to the family of mucins. After glycosylation, it extends 200–500 nm beyond the surface of the cell. Plasma membranes contain the following mucins: leukosialins, gly-

cophorins, sialoglycoproteins and MUC1. MUC1 is known under various names, e.g.: episialin, polymorphic epithelial mucin or epithelial membrane antigen. It constitutes a barrier between the plasma membrane and external environment thereby protecting cells from pathogens, toxins or proteolytic agents [10]. The high density of MUC1 on the cell surface inhibits adhesion. Its anti-adhesion properties result from its unique structure. It has the features of an integral membrane protein. It consists of a repeatable extracellular domain that forms the major part of the core and is extremely immunogenic [11]. Its expression is high prior to implantation. MUC1 is believed to inhibit the interaction between the trophoblast and the apical part of the endometrial epithelium. By forming a barrier for implantation, it causes rejection of poor-quality embryos. MUC1 disappears from epithelial cells beneath and near to the attached embryo, while normal expression persists in neighboring cells [12]. Women with recurrent miscarriages present significantly lower MUC1 levels than the controls [11]. It is hypothetically assumed that healthy fetuses are capable of decreasing the level of this protein during implantation thus becoming its local regulators [11].

Glycodelin A (GdA) can also be considered a potential marker of endometrial function. GdA is believed to play a role during embryo implantation. Thanks to its immunosuppressant properties, a local immune response towards an implanted embryo is inhibited. Infertile women present GdA secretion disorders [13]. GdA belongs to the superfamily of lipocalins. In 2002, the name *glycodelin A* was used for proteins known as placental protein 14 (PP14) or progesterone-associated endometrial protein (PAEP) by a group of authors from Helsinki led by Seppala [14]. The synthesis of GdA by the endometrium increases during embryo implantation. It has been also shown to decrease cytokine production (interleukin-1 and interleukin-2) and its receptor via mitogenic cell stimulation.

Integrins are a family of cell receptors. They react with specific proteins present in the cellular matrix, membrane of other cells or dissolved in body fluids. By cooperating with other membrane receptors, they enable specific cell adhesion as well as aggregation and targeted migration e.g. as an immune reaction of the organism. The regulation of receptor expression occurs at the level of gene promoter via transcription factors [15]. Cells regulate their adhesion properties by integrin expression. Each of integrins is a heterodimer composed of non-covalently associated α and β subunits. To date, 24 different integrins have been found in humans [16].

There are two types of integrins depending on the cycle phase. Their coexistence can determine the occurrence of the window of implantation. Integrins are currently believed to be potential endometrial receptivity markers. Moreover, disorders in the normal model of integrin secretion lead to endometriosis [17]. The transcription of integrins α_4, α_v, β_1 and β_3 is significantly higher in the mid-secretory phase compared with the follicular phase. The greatest increase in expression is noted in subunits α_v and β_3. The more specific subunit β_3 and osteopontin (a ligand of integrins $\alpha_v\beta_3$ – a phosphorylated calcium-binding glycoprotein that is composed of approximately 300 amino acid residues) are found only in the uterine cavity when the endometrium is receptive. Their secretion to the uterine cavity by the endometrial glands during the window of implantation supports the hypothesis concerning the relevance of menstrual cycle-depended proteins in the embryo implantation process [18].

The family of proteins that participate in inflammatory processes and can be found on the surfaces of leukocytes and vascular endothelial cells, includes selectins. L-selectin, also known as CD62L, is a cell adhesion molecule found on lymphocytes and embryos in the preimplantation phase. A blastocyst uses L-selectin to initiate implantation by binding to endometrial ligands composed of oligosaccharide molecules found on the surface of glycoproteins. The absence of L-selectin ligands, called MECA-79, which is believed to be a marker of endometrial receptivity, results in abortion. It is believed that screening for the absence of the aforementioned ligand in the endometrium of women enrolled in *in vitro* programs may help avoid unnecessary failed treatment cycles [19].

Interleukin-1 is the main pro-inflammatory cytokine that participates in hormonal and local reproductive function of the endometrium. The presence of interleukin-1 (IL-1) system on the surface of the endometrium, embryo and placenta suggests that it plays a significant role in the embryo implantation process. The complete IL-1 system is the family of polypeptides consisting of IL-1a, IL-1b, IL-1 receptor and IL-1ra, and can be found in the human endometrium, uterine fluid, embryo and on the surface of the contact site between the embryo and the uterus. Trophoblast cell culture has demonstrated that IL-1 stimulates the secretion of chorionic gonadotropin. Moreover, embryos produce IL-1 only when epithelial cells of the endometrium are added to the culture [20]. The studies quoted above suggest that the blastocyst, by activating IL-1, acts on epithelial cells of the maternal endometrium and regulate integrin subunit secretion.

Interleukin-6 (IL-6) is a classical interleukin of multidirectional action secreted by monocytes and macrophages. The main role of this cytokine is the initiation and regulation of acute inflammatory reaction as well as facilitating development and targeting acquired immune responses. The level of IL-6 expression in the endometrium in the follicular phase is low. Subsequently, a 5–10-fold increase is observed in the mid-secretory phase. It declines again at the end of the secretory phase. This concerns the epithelium and glands, in which the expression is higher than in the stroma [21]. During the window of implantation, IL-

6 receptors can be found not only in the endometrium, but also in the blastocyst. This indicates paracrine/autocrine action of this cytokine during the peri-implantation period. Studies conducted among women with recurrent abortions have shown that IL-6 endometrial m-RNA expression is low in the mid-secretory phase, thus emphasizing the significant role of IL-6 in the embryo implantation process [22].

Furthermore, cadherins also play an important role during implantation. They belong to the superfamily of adhesion proteins – glycoproteins that participate in the interactions between cells via calcium ions (Ca2+). E-cadherin can be found on endometrial cells, and its mRNA level increases in the secretory phase. *In vitro* studies have shown that calcitonin causes a transient rise in intracellular calcium levels, which results in suppressing E-cadherin, at the same time inhibiting cell contact abilities. These results point to calcitonin as an important regulator of the implantation process [22]. It is believed that E-cadherin performs two significant functions in the implantation process. First, it causes adhesion in the initial phase of the process. Second, it inactivates progesterone and calcitonin in the secretory phase thus resulting in the dissociation of epithelial cells, which enables implantation [23]. Endometrial function is tested using EFT (endometrial function test). It consists in immunochemical staining of endometrial biopsies using antibodies against cyclin E. Cyclin E controls the cycle of processes associated with cell division by binding with its kinases and activating them. The prevention of the cell cycle progression is controlled by the p27 cyclin-dependent kinase inhibitor. Cyclin E activity occurs in the cytoplasm of endometrial epithelial cells whilst p27 is active in their nuclei. This activity is high at the beginning of the menstrual cycle. Subsequently, it decreases after cycle day 19. This could be explained by the movement of the nuclei and cyclin E binding to p27, which results in its inactivation. It is thought that the cyclin E marker and its correlation to estrogen and progesterone could be a good tool for the assessment of endometrial receptivity [22].

Currently, genetic markers of endometrial receptivity are being sought for intensively. By using the DNA microarray technique, thousands of genes that could play such a role have been examined. It must be emphasized, however, that results obtained by various researchers are equivocal or even contradicting. Despite this, commercial kits to assess endometrial receptivity have been developed. They are recommended to patients undergoing assisted reproduction procedures with failed attempts to transfer embryos to the uterus. The ERA test has been developed and made available. It is an individual genetic test recommended and useful in the diagnosis of cases with recurrent implantation failures. This test uses the analysis of the expression levels of 238 genes associated with uterine receptivity, and labels the endometrium as either receptive or non-receptive. The test is characterized by the accuracy of predicting the success rate of pregnancy at 62.8% and implantation at 37.9% [24].

Endometrial receptivity is also linked with HOXA-10 and HOXA-11 genes. They belong to the subclass of homeobox genes. Their expression is observed in the glands and stroma of the endometrium throughout the entire menstrual cycle, and their maximal expression is noted during the window of implantation. HOXA-10 gene can be important in the maturation of the endometrium and its decidualization [22, 25].

CONCLUSION

The process whereby the endometrium becomes capable of implantation, i.e. receptive, requires the cooperation and timely coordination of various factors and chemical interactions. The knowledge about receptivity markers and the possibility of practical usage of tests designed for their assessment should, in the near future, become routine management in reproductive medicine.

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