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The role of inflammation for a successful implantation

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Abstract

Problem—Approximately half of all human embryo implantations result in failed pregnancy. Multiple factors may contribute to this failure, including genetic or metabolic abnormalities of the embryo. However, many of these spontaneous early abortion cases are attributed to poor uterine receptivity. Furthermore, although many fertility disorders have been overcome by a variety of assisted reproductive techniques, implantation remains the rate-limiting step for the success of the in vitro fertilization (IVF) treatments.

Results—We, as well as others, have demonstrated that endometrial biopsies performed either during the spontaneous, preceding cycle, or during the IVF cycle itself, significantly improve the rate of implantation, clinical pregnancies and live births. These observations suggest that mechanical injury of the endometrium may enhance uterine receptivity by provoking the immune system to generate an inflammatory reaction. In strong support of this idea, we recently found that dendritic cells (DCs), an important cellular component of the innate immune system, play a critical role in successful implantation in a mouse model.

Conclusion—In this review we discuss the hypothesis that the injury-derived inflammation in the biopsy-treated patients generates a focus for uterine DCs and Mac accumulation that, in turn, enhance the endometrial expression of essential molecules, that facilitate the interaction between the embryo and the uterine epithelium.

Keywords

Dendritic cells; implantation; inflammation; pregnancy

1. The Uterus and Implantation

Embryo implantation, which is an absolute requirement for reproduction, starts with blastocyst apposition to the uterine endometrium, followed by its attachment to the endometrial surface epithelium. Implantation can only takes place in a receptive uterus. In humans the uterus becomes receptive during the mid-secretory phase (days 19-23) of the

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menstrual cycle, commonly known as the window of implantation (WOI). Rodents exhibit a relatively short (4-days) estrous cycle and develop a receptive uterus on day 4 after mating [1].

The uterine endometrium consists of two distinct cellular components, the stromal cells and the cells of the epithelium. The cellular changes during the WOI include the transformation of the fibroblast-like endometrial stromal cells into larger and rounded decidual cells (decidualization) [2], as well as the growth and development of secretory glandules and the emergence of large apical protrusions (pinopodes) and microvilli on the luminal epithelium [3]. In parallel, modulations in the expression of different cytokines, chemokines growth factors, and adhesion molecules take place, as well as vascularization and infiltration of immune cells from the blood to the endometrial tissue [4]. These changes are subjected to regulation by the ovarian steroid hormones, 17-β-estradiol and progesterone. [5, 6].

The modulated expression of the above-mentioned molecules at the WOI provides circumstantial evidence for their role in this process. However, the association of some of these specific endometrial genes with impaired fertility in humans has not been consistent [5, 6]. Many efforts have been invested in order to identify specific molecules that characterize receptive endometrium. Different “omics” technologies such as genomics (global microarray analysis)7-13, proteomics 14 and secretomics 15-17, performed in the last decade revealed a large number of genes expressed differentially in human endometrium during the WOI. Moreover, based on a customized microarray, a new assay, endometrial receptivity array (ERA), has been recently developed for prediction of endometrial receptivity 18, however its use in the clinic still needs to be further validated.

2. Tissue Repair and Implantation

We have demonstrated that endometrial biopsies taken during the spontaneous cycle that preceded the in vitro fertilization (IVF) and embryo transfer (ET) treatment more than doubled the rates of implantation, clinical pregnancies and live births [19]. Such favorable influence of the biopsy-treatment on IVF outcome was later observed by other clinics worldwide 20-25, and further confirmed by two independent meta-analyses 26, 27. Moreover, Gibreel et al, 2013 have recently shown for the first time that biopsy treatment not only increases the chance of implantation in IVF patients but also positively affects the outcome of spontaneous pregnancies 28.

Mechanical manipulation, which is associated with formation of decidua is not a new phenomenon. In 1907, Leo Loeb [29], first reported that scratching the guinea-pig uterus during the progestational phase of the estrous cycle provoked a rapid growth of decidual cells. Later experiments showed that decidua formation in pseudopregnant rodents could be induced by other forms of local injury, such as suturing the uterine horn [30] and intrauterine injection of oil [31]. These early observations in rodents, combined with our recent findings in human patients, suggest that local injury of the endometrium facilitates successful implantation. Additionally, albeit indirect, evidence to support the beneficial effect of endometrial injury on successful implantation comes from the observation that scar tissue from previous endometrial surgery (or Cesarean section) becomes an attractive site of

implantation [32]. Taken together, these reports suggest that it is possible that the success of implantation is secondary to the development of an injury-induced inflammatory reaction [33].

3. Cytokines, immune cells and Implantation

A high level of the pro-inflammatory T helper (Th)-1 and cytokines (IL-6, IL-8, TNFα) characterizes early implantation [34-33, 35, Van Sinderen, 2013 #567, 36, 37]. We previously demonstrated that endometrial biopsy up-regulates the expression of different pro-inflammatory cytokines such as macrophage inflammatory protein (MIP)1B, TNFα, GROα, osteopontin (OPN) and IL-15 as well as the abundance of the specific immune cells, macrophages (Mac) and dendritic cells (DCs) [38]. We further showed that the levels of these cytokines and immune cells positively correlate with the pregnancy outcome of the IVF patients [38]. A recent study showed that increase in the expression of interferon-inducible protein-10 (IP-10) and TNFα in endometrial aspiration of IVF patients is associated with successful implantation[39]; supporting the concept that cytokines and chemokines are critical for the success of implantation. It is important to note that in addition to their classical role to attract and activate immune cells, cytokines such as IL-6, MIP-1B, CX_3CL1 and IP-10 are directly involved in the implantation process by attracting human trophoblast cells [40-42]. The role of IP-10 in regulation of blastocyst migration and apposition has been also confirmed *in vivo* in the mouse [43]. These cytokines are secreted by the endometrial cells as well as by the immune cells that are recruited to the site of implantation. Indeed the utero-placental unit of human and mice was shown to be richly populated with hematopoietic cells. Of these, 65-70% are uterine-specific natural killer (NK) cells, and 10-20%, are antigen presenting cells (APC) such as macrophages (Mac) and dendritic cells (DCs)[44-50]. Circulating NK cells in the peripheral blood are cytotoxic, however, upon their infiltration into the endometrium, they undergo differentiation into uNK cells, losing their killing activity [51]. This change in their characteristics is mediated by IL-15, secreted by DCs and endometrial cells, and by TGF-β1, that is secreted by Mac [52-55]. Decidual NK cells (dNK) have a role in regulating trophoblast invasion by the production of IL-8 and IP-10 chemokines [44]. Moreover, these cells were also demonstrated to trigger endometrial stromal cells to produce chemokines, IL-8, CCL8 and CXCL1, that act synergistically with uNK to induce trophoblast migration [56]. In support to these findings, a recent study that applied a new method of morphometric image analysis, demonstrated a higher density of dNK cells and Mac in close proximity to the invasive trophoblast in human tissue fragments derived from first trimester placentation sites[57]. Decidual NK cells are also potent secretors of an array of angiogenic factors that induce vascular growth that is essential for the establishment of an adequate decidua [58]. DCs are a heterogenous population of cells that initiate and coordinate the innate adaptive immune response. These cells accumulate in the pregnant uterus prior to implantation and stay in the decidua throughout pregnancy [45, 59, 60]. Several lines of evidence point to a pivotal role of APC cells in shaping the cytokine profile at the maternal–fetal interface [59, 61, 62]. Furthermore, a previous study from our laboratory showed that depletion of uterine DCs (uDCs) cells resulted in a severe impairment of implantation and led to embryo resorption [63]. However, the effect observed in our study was not related to tolerance but rather to successful decidualization. In agreement with our findings, another study showed that therapy with DCs significantly decreased the spontaneous resorption rate in a mouse

model [64]. These studies suggest that, in addition to their involvement in the immune response, uDCs also play some tropic role in regulating pregnancy. It was shown that uterine DCs and Mac secrete, both, pro-inflammatory and anti-inflammatory cytokines, by which they were suggested to balance the endometrial Th1/Th2 cytokines that control endometrial tissue remodeling and growth [50, 61, 65]. Indeed, Mac have been shown to regulate endometrial remodeling and clearance of apoptotic trophoblast cells during trophoblast invasion [47, 66]. Furthermore, It was recently demonstrated that Mac also directly regulate the expression of endometrial epithelial fucosyltransferases, FUT1 and 2, enzymes that are involved in synthesis of fucosylated ligands, required for embryo attachment in cells [67, 68]

The immune infiltrate, that plays a central role in the process of tissue renewal and differentiation, may also participate in the development of a receptive endometrium in biopsy-treated patients. In addition to their immediate influence, recruitment of cells of the immune system to the site of injury may create some “tissue memory” facilitating implantation in the following cycle of treatment. In fact, monocyte precursors of macrophages and DCs are known to be recruited to injured sites and provide essential beneficial effects during wound healing. These cells are long lived and reside in some tissues for months during which time they can differentiate into tissue-resident macrophages or DCs [69].

Accumulating evidence suggests that intrauterine administration of autologous freshly isolated peripheral blood mononuclear cells (PBMC) also improves embryo implantation. It was recently shown that this treatment increases implantation rates in patients with three or more IVF failures [70]. Previous studies showed that intrauterine administration of *in vitro* hCG pre-activated PBMC also increased implantation rates in patients with repeated implantation failure [71, 72]. A recent study in mice showed that increased endometrial receptivity following hCG-treated PBMC administration is associated with elevated endometrial expression of LIF and VEGF. This effect has been suggested to be mediated by hCG-induced secretion of IL-1β and TNFα by the PBMC [73]. It was also suggested that PBMC facilitates implantation by increasing epithelial cell adhesive properties [74]. Recently we reported the in vitro effect of hCG on trophoblast-epithelium interaction by decreasing MUC16 expression and inducing OPN secretion, two factors which are thought to be relevant for implantation as we discussed below[75].

4. The Trophoblast-Lumen Epithelium Synapse

As the blastocyst travels from the fallopian tube to the uterine cavity, the surface epithelium of the uterus functions as the first contact responsible for adequate attachment of the trophectoderm to the epithelium and the subsequent trophoblast invasion and placentation. When a mammalian blastocyst enters the uterine cavity, the surface epithelium of the uterus is covered by molecules, such as Mucin 1 (MUC1) carbohydrates that prevent the attachment of the highly adhesive blastocyst to an improper site. Indeed, in the human endometrium MUC1 is up-regulated during the implantation period [76]. It prevents the adhesion of the blastocyst, except for the precise spot in which MUC1 is shed by specific metalloproteinases, secreted by the blastocyst.[76-82]. Another member of the mucin family, MUC16, was also demonstrated as a critical molecule that has to be dow-regulated in order

to enable the adhesion of the trophoblast cells to the epithelium.[83]. Alternatively, embryo-endometrium interaction is stabilized by adhesion molecules [84]. Adhesion of the trophoblast is mediated by integrins that are expressed by both, the endometrium and the blastocyst. Integrins bind to their ligands, fibronectin, vitronectin, thrombospondin, and osteopontin (OPN) that serve as a bridge between the luminal uterine epithelium and the blastocyst [84]. Our previous study revealed that biopsy-induced improvement in the implantation rates of the IVF patients is associated with the increased endometrial expression of OPN [38]. This molecule is indeed most highly expressed in the endometrium during its receptive phase [38, 85], Functional blocking of endometrial OPN and its receptor integrin (ITG) αvβ3 by injection of specific antibodies significantly reduced the number of implantation sites in the mouse [86, 87]. Furthermore, pre-treatment of epithelial cells with ITGβ3 siRNA *in vitro*, prevented the attachment of blastocysts to these cells [88].

There are four ways by which blastocysts binding to the epithelium may be enhanced i) stored adhesion molecules are rapidly moved to the cell surface; ii) inflammation-induced expression of new adhesion molecules; iii) increased affinity of specific molecules following initial cell contact; and iv) reorganization of adhesion molecules on the surface epithelium as well as local degradation of anti-adhesive molecules (Fig. 1). Taking all the above-mentioned information into consideration, we hypothesize that either of these possibilities or their combination can represent the response of the endometrial epithelium to DCs recruited to the site of implantation (Fig. 2).

It is important to mention that the expression of the above discussed adhesive and non-adhesive molecules is under the regulation of the pro-inflammatory cytokines secreted by endoemtrial cells as well as by the recruited immune cells [81, 89, 90]. This, in fact, supports the notion that inflammation plays a crucial role in the acquisition of endometrial receptivity. We have developed an in vitro model of uterine implantation that allow us to furhter elucidate the role of inflammatory factors in the process of inplantation [91]

Summary and Relevance

Many fertility disorders have been overcome by a variety of assisted reproductive techniques. Nevertheless, embryo implantation remains the rate-limiting step for the success of IVF. Attempts to maximize the chance of successful implantation include the transfer of more than a single embryo. This strategy results in a high incidence of multiple gestations, which is related to the number of embryos transferred. The rate of multiple gestations after IVF, in both Europe and the USA, is 26.4 and 35.4 percent, respectively and multiple gestation pregnancies are the major cause for the increased risks of potential death and premature birth in IVF patients. Furthermore, restricted fetal growth, increased incidence of congenital malformations and greater likelihood of maternal complications has been reported as a consequence of multiple birth pregnancies. Deciphering the components that are associated with improved implantation can be employed for identifying new therapeutic targets and developing novel means to extend uterine receptivity. Furthermore, once an improved rate of successful implantation is achieved it may serve as a strong incentive for the use of single-embryo transfers, avoiding multiple birth pregnancies and their subsequent, often severe implications. Moreover, increasing the rate of pregnancy in IVF/ET programs

will reduce the number of repeated cycles of treatment, thus lowering the possible risks associated with massive exposure to gonadotropins. Equally important, the information generated from studies associated with the inflammatory response during implantation will potentially define hitherto unavailable markers that will serve to predict low chances for successful implantation further recommending that IVF may not always be the immediate solution.

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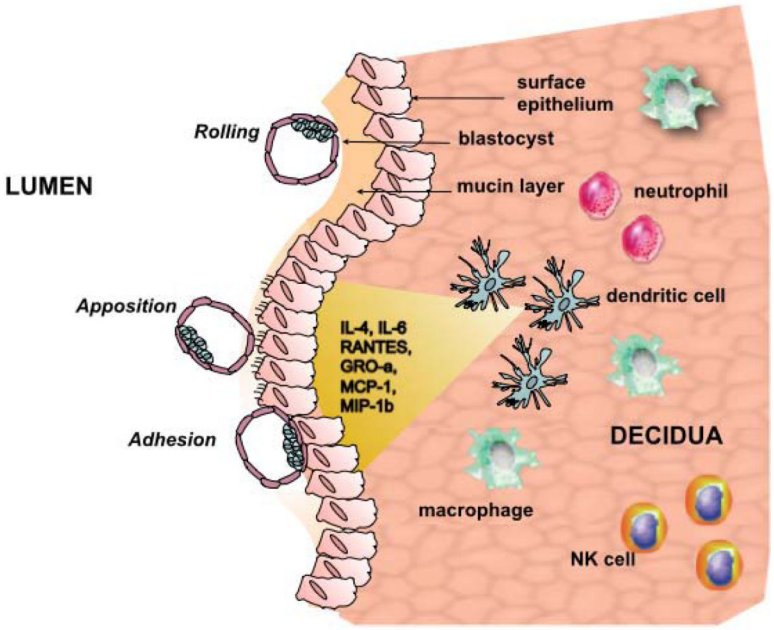

Figure 1.

Dendritic Cells and Macrophages create an inflammatory gradient, which affects the epithelium to form the mucin layer and increase the expression of ligands for adhesion molecules by the blastocyst. The inflammatory gradient allows the apposition and adhesion of the blastocyst to the epithelium and promotes implantation.

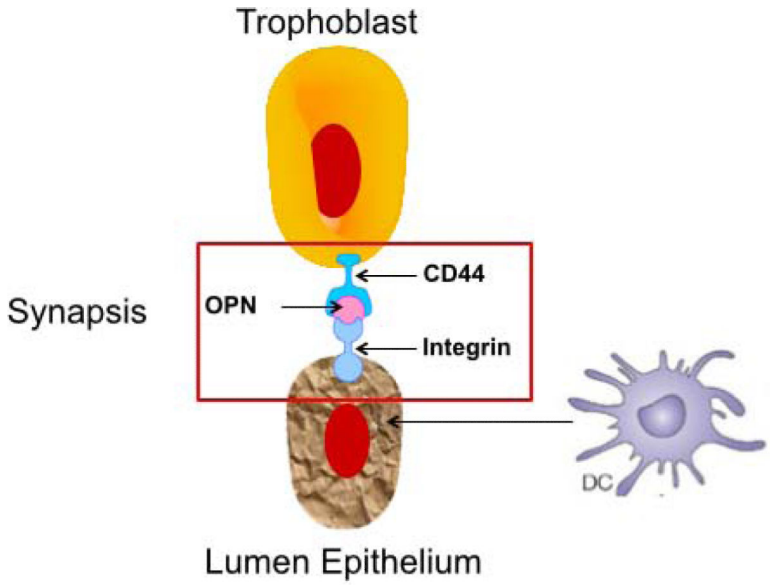

Figure 2.
Model of trophoblast-epithelium synapsis. Potential role of OPN as a bridge for CD44 on the trophoblast and integrins in the epithelium.