JCI The Journal of Clinical Investigation

Uterine DCs are essential for pregnancy

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J Clin Invest. 2008;118(12):3832-3835. https://doi.org/10.1172/JCI37733.

Commentary

Successful embryo implantation requires complex interactions between the uterus and embryo, including the establishment of maternal immunologic tolerance of fetal material. The maternal-fetal interface is dynamically populated by a wide variety of innate immune cells; however, the relevance of uterine DCs (uDCs) within the decidua to the success of implantation has remained unclear. In this issue of the *JCI*, Plaks et al. show, in a transgenic mouse model, that uDCs are essential for pregnancy, as their ablation results in a failure of decidualization, impaired implantation, and embryonic resorption (see the related article beginning on page 3954). Depletion of uDCs altered decidual angiogenesis, suggesting that uDCs contribute to successful implantation via their effects on decidual tissue remodeling, including angiogenesis, and independent of their anticipated role in the establishment of maternal-fetal tolerance.

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Uterine DCs are essential for pregnancy

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Successful embryo implantation requires complex interactions between the uterus and embryo, including the establishment of maternal immunologic tolerance of fetal material. The maternal-fetal interface is dynamically populated by a wide variety of innate immune cells; however, the relevance of uterine DCs (uDCs) within the decidua to the success of implantation has remained unclear. In this issue of the *JCI*, Plaks et al. show, in a transgenic mouse model, that uDCs are essential for pregnancy, as their ablation results in a failure of decidualization, impaired implantation, and embryonic resorption (see the related article, doi:10.1172/JCI36682). Depletion of uDCs altered decidual angiogenesis, suggesting that uDCs contribute to successful implantation via their effects on decidual tissue remodeling, including angiogenesis, and independent of their anticipated role in the establishment of maternal-fetal tolerance.

Placental viviparity, a mode of reproduction during which nutrients are supplied to the embryo directly from the mother via the placenta, poses a number of challenges. The first is the requirement for coordinated development of maternal and fetal tissue, while the second, in mammals, demands maternal immunologic tolerance of the fetus, which expresses foreign transplantation antigens. This latter requirement poses a significant immunological challenge, because mechanisms of graft rejection need to be suppressed so as to avoid fetal loss, while at the same time, an adequate defense against pathogens must be maintained. Original proposals regarding how this balance is achieved suggested that the fetus is immunologically inert (1). But this contention was soon shown to be incorrect, and it is now appreciated that pregnancy involves complex immune regulation so as to prevent cytotoxic T cells from responding to fetal antigens, while simultaneously maintaining immunity at the maternal-fetal interface (1). In fact, early observations that the uterine environment is rich in hematopoietic growth factors/cytokines (whose expression in many cases is regulated by the ovarian sex steroid hormones 17β-estradiol and progesterone), coupled with the observation of the dynamic recruitment of diverse innate immune cells, led to the proposal that these immune cells play an important role in decidual and placental development (2, 3). Among the earliest growth factors expressed in the uterus are GM-CSF and CSF-1, which regulate the myeloid system (4, 5). Levels of CSF-1 synthesized by the uterine epithelium are elevated at the time of implantation and continue to climb dramatically throughout the process of placentation (4). CSF-1 has been found in all mammalian species tested (3), and this growth factor is the major regulator of the mononuclear phagocytic lineage and controls macrophage proliferation, migration, viability, and function as well as having a significant role in DC development (6). Macrophages and DCs both accumulate after implantation around the decidua and in the uterus throughout pregnancy (7, 8). These antigen-presenting cells could be detrimental if they were to present fetal antigens to T cells, so the prevailing view is that these antigen-presenting cells are trophic and/or tolerogenic (9).

Nonstandard abbreviations used: DT, diphtheria toxin; sFLT1, soluble FMS-like tyrosine kinase 1; uDC, uterine DC; uNK cell, uterine NK cell.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* doi:10.1172/JCI37733.

Ablation of uterine DCs blocks decidualization

The study by Plaks et al. (7) in this issue of the *JCI* reports that uterine DCs (uDCs) are

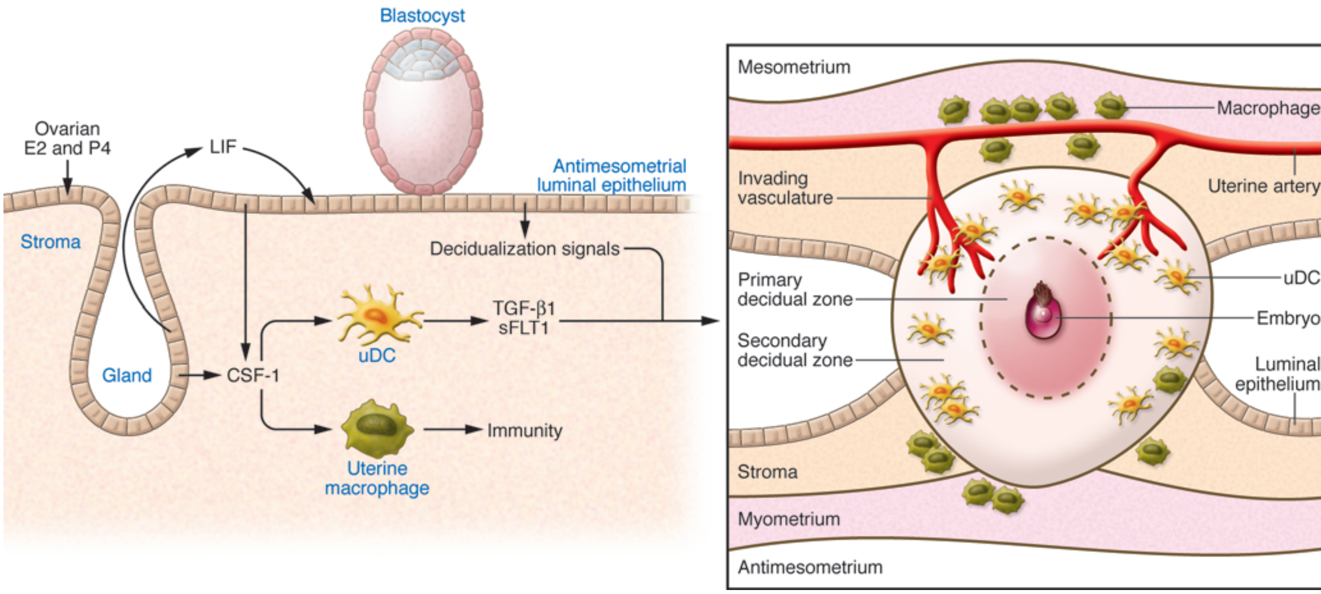

Figure 1
uDCs regulate decidualization. At the beginning of pregnancy, the ovarian steroid hormones 17β-estradiol (E2) and progesterone (P4) stimulate synthesis of growth factors from the uterine epithelium, including CSF-1 and leukemia inhibitor factor (LIF). LIF, acting on the luminal epithelium, is essential for blastocyst implantation and subsequent decidualization. CSF-1 recruits and regulates uterine macrophages, while also influencing the biology of uDCs. In addition, CSF-1 acts on decidual cells and trophoblastic cells (not shown). Macrophages play a largely immune role at this stage, while, as Plaks et al. (7) show in their current study in this issue of the *JCI*, uDCs are required for efficient decidualization. This decidualization process involves transformation of stromal cells into epithelioid-type cells at the site of blastocyst implantation on the antimesometrial side of the uterus. The decidual cells grow in an arc, via rapid proliferation and transformation, to surround the implanting embryo. After implantation is complete, the blastocyst is entirely surrounded by a primary decidual zone of polyploid cells and a secondary diploid decidual zone. Vascularization commences on the mesometrial side of the uterus via the growth of vascular sprouts from the uterine artery into the decidua, which results in vessels with large, dilated lumens through which maternal blood bathes the decidual nodule. As Plaks et al. report, uDCs located in the outer layers of the decidua regulate this vascularization in part through their synthesis of TGF-β1 and sFLT1. Ablation of uDCs results in a failure of decidual growth and resorption of the embryo, showing that uDCs are essential for a successful pregnancy.

required for successful embryo implantation and decidualization in mice (Figure 1). The authors used a suicide gene ablation approach to specifically delete uDCs during embryo implantation in these animals. As part of this approach, the human diphtheria toxin receptor (DTR) was expressed from the CD11c promoter, which made cells expressing the receptor uniquely sensitive to diphtheria toxin (DT); mice do not have the DTR and are thereby resistant to DT. Since CD11c is restricted to DCs, these cells were rapidly ablated with little to no ablation of other hematologic cells. The ablation of uDCs during implantation resulted in a failure of decidualization and embryo resorption. This effect was specific to the uterus and did not involve the embryo, since uDC ablation also blocked decidualization in an artificially induced model of decidualization in the absence of the embryo. Furthermore, this was a local effect and not secondary to a systemic effect, as administration of DT to one uterine horn resulted in retarded decidualization, while the contralateral control horn was unaffected. In addition, uDC ablation resulted in failed decidualization in both allogeneic and syngeneic pregnancies. These data indicate that uDCs are essential to implantation and decidualization, and this requirement did not have an immunological component, but rather represented trophic activities of uDCs.

Decidual angiogenesis is regulated by uDCs

These studies raise the question, By what mechanisms do DCs affect decidualization? One of the earliest events in the decidual response is an increase in vascular permeability, induced by the rapid expression of the angiogenic factor VEGF upon embryo attachment to a suitably hormone-primed uterus. This is in fact the basis for the earliest test of implantation, called *blue spotting*, which is the result of extravasation of i.v. administered pontamine blue dye at these sites of vascular permeability immediately below the attached blastocyst. After this increase in vascular permeability, there is rapid decidual cell proliferation and decidual transformation of the underlying stroma, giving rise to epithelioid-type cells that surround the invading blastocyst. These cells form the primary decidual zone, which is in turn surrounded by a diploid secondary decidual zone (Figure 1). After the decidua is formed, there is extensive vascularization via sprouting of the uterine artery at the mesometrial side (site of future placenta), which results in the formation of very dilated vessels and the bathing of the implantation nodule with maternal blood. Using sophisticated dynamic macromolecular contrast-enhanced MRI–assisted studies following partial uDC ablation that allowed some decidual response compared with controls, Plaks et al. show that these early vascular events were significantly perturbed following uDC ablation (7). Indeed, the data suggest that uDC depletion delayed the angiogenic response, increased vascular permeability, and inhibited blood vessel maturation.

In mice, uDCs are restricted to the outer decidual zones (Figure 1) and are often found associated with blood vessels, consistent with a role in angiogenesis. Taking a

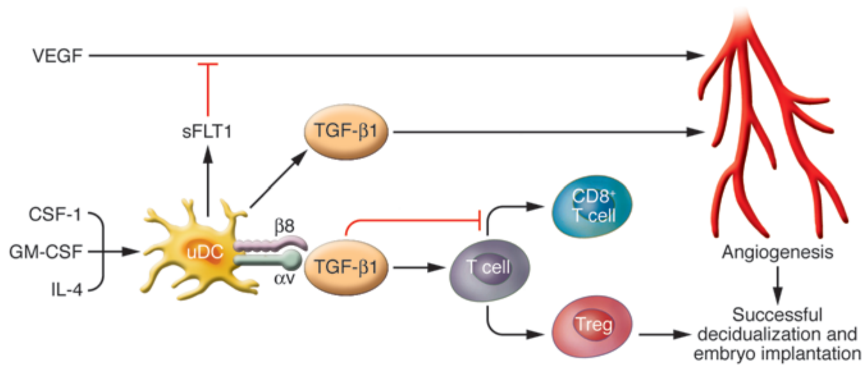

Figure 2
Proposed roles of uDCs in the regulation of angiogenesis and T cell action at the maternal-fetal interface. During pregnancy, monocytes are recruited to the uterus, where they differentiate into mature tolerogenic cells such as uDCs, under the influence of CSF-1, GM-CSF, and other cytokines (usually IL-4, although this cytokine has not been found in the uterus). uDCs produce sFLT1 and TGF-β1, which act to maintain the intricate balance of vascular development: sFLT1 modulates the actions of VEGF, and TGF-β1 influences endothelial cell viability and vascular maturation. This fine-tuning of angiogenesis is required for decidualization and embryo implantation. In addition, other studies have shown that TGF-β1 is presented by DCs at their surface on αvβ8 integrin. TGF-β1 suppresses cytotoxic $CD8^+$ T cell function and promotes the development of Tregs. These data suggest that in addition to their role in decidual development, as shown in the present study by Plaks et al (7), uDCs also play a role in immunoregulation. Together, these dual functions of uDCs contribute to successful implantation and the progression of an allogeneic pregnancy. This whole process is further coordinated during implantation and decidualization by the uterine synthesis of the growth factors VEGF and CSF-1 under the control of the ovarian hormones E2 and P4 (see Figure 1), which are the master regulators of pregnancy.

targeted gene approach, the authors found that soluble FMS-like tyrosine kinase 1 (sFLT1) — a soluble form of VEGFR1 — was expressed by uDCs (7). This molecule, by acting as a trap, opposes the actions of VEGF, and Plaks et al. suggest that the absence of sFLT1 results in excessive VEGF action and a disruption to the fine-tuning of angiogenesis. Such an interpretation, although not tested experimentally in the current study by Plaks et al., is consistent with the results obtained following inhibition of VEGF by neutralizing antibodies, which was also shown to prevent decidualization and pregnancy in rodents (10). These authors also showed that uDCs express TGF-β1 and that this molecule was depleted following uDC ablation (7). Among its many activities, TGF-β1 is involved in endothelial cell survival and vascular maturation. Such functions are further consistent with a role of uDCs in angiogenesis. Interestingly, in other contexts, DCs synthesize and sequester TGF-β1 on αvβ8 integrin and as a consequence suppress T cell responses and promote the development of Tregs (11). Tregs are central to the establishment and maintenance of maternal immune tolerance during pregnancy, as their ablation results in loss of allogeneic pregnancies (12). This action may thus couple the role of uDCs in decidual formation through their action on angiogenesis (7) with their tolerogenic role in preventing immune rejection of the fetus, thus resulting in successful implantation free from immune attack (Figure 2).

Placentation is regulated by the interplay of innate immune cells

The uteroplacental unit of mice and humans is richly populated with hematopoietic cells such as macrophages, uterine NK (uNK) cells, and uDCs. These cells are dynamic in their recruitment and location and are regulated by the local synthesis of hematopoietic cytokines such as GM-CSF, CSF-1, IL-11, IL-15, and TNF-α, often under the control of estrogen and progesterone. While these cells have roles in the immune response, the current study (7) as well as previous ones (13, 14) establish that they are also trophic, in that all three cell types play important roles in regulating pregnancy. Macrophages, for example, regulated by CSF-1, play a major role in the immune response against pathogens in the decidua (15) and are also involved in the ripening of the cervix during parturition (14). IL-11 recruits immature uNK cells, while at the same time this cytokine also regulates the development of the mesometrial decidua (16, 17). IL-15, expressed later, regulates the maturation of uNK cells that remodel the spiral arteries and are involved in placental angiogenesis, even though these cells are not essential for pregnancy, at least in mice (13, 18, 19). The current study by Plaks et al. identifies uDCs as another essential cell type in pregnancy by showing that they are necessary for decidual formation, in part through their effects on vascularization but also probably other actions directly on decidual cells. These trophic roles for recruited hematopoietic cells in decidual and placental development further reinforce the view that a major role of migratory hematopoietic cells of the innate immune system is in tissue development and these trophic functions are often sequestered in pathological contexts such as cancer (20).

Given these profound effects of uDC ablation on decidual angiogenesis (7), it is possible that perturbations in normal uDC activity could reduce the fitness of the fetus, for example in such conditions as preeclampsia, a major cause of low fetal birth weight as well as maternal and fetal morbidity in women. This condition is thought to result from limited trophoblastic invasion and poor decidual and placental vascularization and to be initiated early in fetal development. Indeed, ratios of circulating angiogenic and antiangiogenic factors are predictive of the onset of preeclampsia, with the serum concentration of sFLT1 being an important determining factor that predicts the preeclamptic condition (21). The new data reported in the study by Plaks et al. (7) indicating that uDCs are a major contributor to sFLT1 synthesis at the uteroplacental interface will now focus further research on the function of these cells and their possible involvement in the etiology of preeclampsia.

Acknowledgments

The author is the Louis Goldstein Swan Chair in Women's Cancer Research, and his research is supported by NIH grants HD30820, HD050614, CA131270, and CA100324 and by a grant to the Cancer Center from the National Cancer Institute (P30-13330).

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Tumor metabolism: cancer cells give and take lactate

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Tumors contain well-oxygenated (aerobic) and poorly oxygenated (hypoxic) regions, which were thought to utilize glucose for oxidative and glycolytic metabolism, respectively. In this issue of the *JCI,* Sonveaux et al. show that human cancer cells cultured under hypoxic conditions convert glucose to lactate and extrude it, whereas aerobic cancer cells take up lactate via monocarboxylate transporter 1 (MCT1) and utilize it for oxidative phosphorylation (see the related article, doi:10.1172/JCI36843). When MCT1 is inhibited, aerobic cancer cells take up glucose rather than lactate, and hypoxic cancer cells die due to glucose deprivation. Treatment of tumor-bearing mice with an inhibitor of MCT1 retarded tumor growth. MCT1 expression was detected exclusively in nonhypoxic regions of human cancer biopsy samples, and in combination, these data suggest that MCT1 inhibition holds potential as a novel cancer therapy.

The pioneering work of Peter Vaupel and his colleagues established that the partial pressure of oxygen (pO_2) within human cancers is frequently much lower than that of the surrounding normal tissue and that intratumoral hypoxia is associated with an increased risk of local spread, metastasis, and patient mortality (1). Rakesh Jain's laboratory demonstrated that in mouse tumor xenografts, the mean pO_2 and pH declined as distance from the nearest blood vessel increased (2), reflecting the switch from oxidative to glycolytic metabolism that occurs in response to reduced O_2 availability.

This metabolic reprogramming is orchestrated by HIF-1 through the transcriptional activation of key genes encoding metabolic enzymes, including: *LDHA*, encoding lactate dehydrogenase A, which converts pyruvate to lactate (3); *PDK1*, encoding pyruvate dehydrogenase kinase 1, which inactivates the enzyme responsible for conversion of pyruvate to acetyl-CoA, thereby shunting pyruvate away from the mitochondria (4, 5); and *BNIP3*, which encodes a member of the BCL2 family that triggers selective mitochondrial autophagy (6) (Figure 1). In addition, HIF-1 transactivates *GLUT1* (7) — which encodes a glucose transporter that increases glucose uptake to compensate for the fact that, compared with oxidative phosphorylation, glycolysis generates approximately 19-fold less ATP per mole of glucose — and genes encoding the glycolytic enzymes that convert glucose to pyruvate (3). The extracellular acidosis associated with hypoxic tumor cells is due to both increased H^+ production and increased H^+ efflux through the HIF-1–mediated transactivation of: *CA9*, which encodes carbonic anhydrase IX (8); *MCT4*, which encodes monocarboxylate transporter 4 (9); and *NHE1*, which encodes sodium-hydrogen exchanger 1 (10).

Metabolic symbiosis

In this issue of the *JCI*, the elegant article by Sonveaux, Dewhirst, et al. makes a major contribution to the field of cancer biology (11).

Nonstandard abbreviations used: LDH, lactate dehydrogenase; MCT, monocarboxylate transporter.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* doi:10.1172/JCI37373.