MRI of Perturbed Decidual Angiogenesis - a Characteristic of an Embryo Implantation Failure Associated with the Conditional Depletion of Uterine Dendritic Cells

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Introduction:

Implantation is a critical stage in the establishment of pregnancy. Embryo implantation failure is clinically relevant to recurrent pregnancy loss and low success of in vitro fertilization. Many of these early abortion cases are thought to result from poor uterine receptivity. Angiogenesis is a prerequisite for uterine receptivity, as the endometrium transforms into the decidua. Angiogenesis in mouse implantation site (IS) is primarily characterized by increased vascular permeability on embryonic day (E) 4.5 (right after implantation) followed by a rise in blood volume on E5.5 that can be non-invasively detected by dynamic macromolecular-contrast enhanced MRI [1]. $CD11c^{high}$ dendritic cells (DC) are the most potent inducers of primary immune responses and represent around 5-10% out of all hematopoietic uterine cells [2]. DC accumulate in the pregnant uterus prior to implantation and remain through most of the pregnancy in the decidua, both in humans and rodents [2]. Here we investigated the role of uterine DC (uDC) in embryo implantation, using transgenic mice in which DC can be ablated using Diphtheria toxin (DTx) [3]. We report that uDC are required for embryo implantation and critically involved in decidual angiogenesis as revealed by macromolecular contrast enhanced 3D-MRI.

Materials and Methods:

Animals: The $CD11c:DTR$ mouse model allows the conditional ablation of $CD11c^{high}$ DC by DTx administration [3]. Pregnant mice were intraperitoneally injected with DTx on E.3.5 and analyzed on E4.5 and E5.5. DTx injection caused depletion of $CD11c^{high}$ DC within 8 hours, which lasted for at least two days [3], thus covering the implantation window (E4-4.5 till E5.5). Control groups included E3.5 $CD11c:DTR$ female mice injected with PBS and E3.5 WT mice injected with DTx.

MRI experiments: Animals were placed in a 4.7T horizontal Bruker Biospec (Germany) spectrometer and a whole body excitation coil was used. Contrast agent: biotin-BSA-GdDTPA was prepared as reported [4]. IS were retrieved and examined for the depletion of uDC and embryo status.

3D-GE: A series of images with 15, 5, 30, 50, 70 degrees flip angles were acquired to determine the precontrast R1 (TR 10ms, TE 3.6ms, 2 averages, spectral width 50000Hz, matrix 128x128x64 (zero filled to 128), FOV 4x4x4 cm). Dynamic post contrast images were acquired with a 15 degrees flip angle and animals were followed for 24min after intravenous (i.v.) administration of biotin-BSA-GdDTPA via the tail vein.

MRI data analysis: Mean biotin-BSA-GdDTPA concentrations were derived from the precontrast R1 and pre- and postcontrast 3DGE-mean signal intensities at ROIs of IS were used for derivation of blood volume fraction (fBV) and permeability surface area product (PS; contrast agent extravasation rate to the interstitial space) [1].

Histology: IS were retrieved and stained with Eosin and Hematoxilin to examine tissue morphology and with avidin-FITC to visualize the MR contrast agent.

Results:

E5.5 uDC- depleted (+DTx) IS exhibited significantly reduced decidua versus the control (Fig. 1A). Embryos in the uDC- depleted IS were detached from the decidua and under-developed (Fig. 1A inserts). UDC-depleted IS were significantly smaller and showed reduced contrast enhancement relative to control IS, as shown by the maximal intensity projections at 24 min post MR contrast injection (Fig. 1B). The blood volume fraction (fBV) calculated using the MRI data was significantly lower in uDC- depleted implantation sites (Fig. 1C). However, despite the significantly reduced blood volume and vessel density, (and thus also vessel surface area), the permeability surface area product (PS) of uDC-depleted implantation sites was similar to that of control IS (Fig. 1D). This suggested that the trans-capillary leak of plasma proteins was higher in E5.5 uDC-depleted IS. The MRI results demonstrating impaired angiogenesis were corroborated by fluorescence analysis of the i.v. injected biotinylated MR contrast agent stained with avidin-FITC. As shown in Fig. 1E, on E5.5 at 3 min post contrast, blood vessels observed in the uDC-depleted IS were mostly from the myometrium. In contrast, in the control, smaller blood vessels characteristic of the decidua were detected within the IS. At 15min, only a minor part of the overall contrast material had extravasated from the blood vessels in uDC- depleted sites, as compared to the control group. Quantitative analysis of the E5.5 data (Fig. 1F), revealed a significant reduction in the fluorescence of the decidual part in both 3 and 15 min of the uDC- depleted versus control IS. Comparable to the MRI data, uDC- depleted IS exhibited reduced vessel density (as estimated by 3 min post contrast) and similar permeability (as indicated by similar change over time between 3 and 15 min). Similar results were obtained for E4.5 (not shown) although less pronounced, since the decidua is less extended at this time point.

Discussion:

Contrast enhanced 3DGE-MRI using biotin-BSA-GdDTPA was used to evaluate an implantation failure caused by depletion of uDC in transgenic mice. MRI revealed that uDC- depleted uteri exhibited impaired decidual angiogenesis characterized by decreased blood volume and increased vascular permeability, opposing the trends of these parameters in normal implantation along with impaired proliferation and differentiation examined by immuno-histochemistry (not shown). Along with other data (not shown), our results argue against a role of uDC in tolerance establishment towards the embryo as uDC-depletion caused resorptions also in syngeneic and T cell-deficient pregnancies, and even during artificial decidualization in the absence of an embryo. Collectively, our data suggest a novel non-immune role for uDC in tissue remodeling directly linked to the decidualization process mediating uterine receptivity.

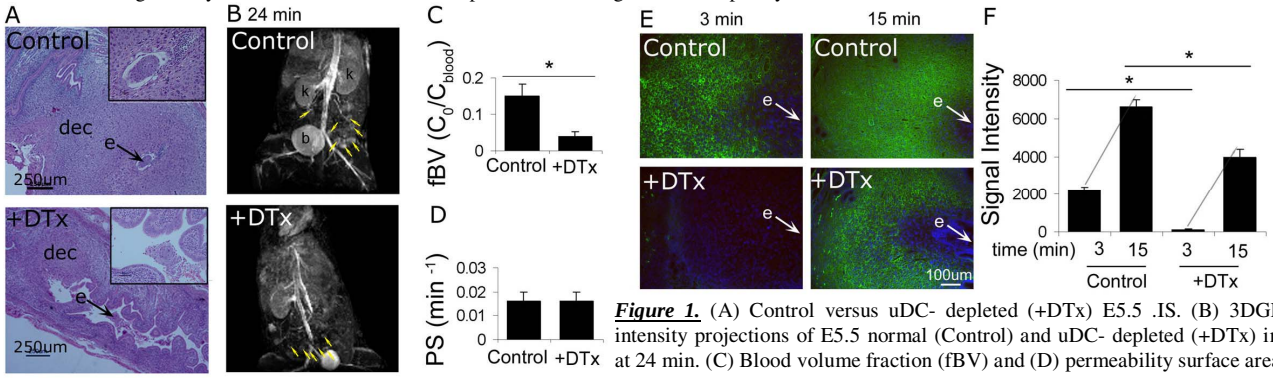

Figure 1. (A) Control versus uDC- depleted (+DTx) E5.5 .IS. (B) 3DGE-MRI maximal intensity projections of E5.5 normal (Control) and uDC- depleted (+DTx) implantation sites at 24 min. (C) Blood volume fraction (fBV) and (D) permeability surface area product (PS) of normal versus uDC- depleted E5.5 IS (Control - 3 mice, 10 IS; +DTx- 2 mice, 6 IS, $P_{fBV} = 0.03$, $P_{ps} = 0.89$). (E- F) E5.5 IS were retrieved after 3 and 15 min post biotin-BSA-GdDTPA injection and stained with avidin-FITC (2 mice, 3 implantation sites per time point were used, $P_{3min} = 1.6 \times 10^{-5}$, $P_{15min} = 0.002$). Grey trend line indicates permeability. Yellow arrow- IS, white or black arrows- embryo location, b- bladder, k- kidney, e- embryo, dec- decidua.

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