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Bi-directional Cell Trafficking Between Mother and Fetus in Mouse Placenta

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Abstract

It is now well established that cells are exchanged between mother and fetus during gestation. It has been proposed that some of these exchanges take place in the placenta, but it has never been demonstrated. Here, we made use of EGFP (Enhanced Green Fluorescent Protein) transgenic mice to precisely visualize the juxtaposition of maternal and fetal tissues at the implantation site, as well as to describe the bi-directional cell trafficking between mother and fetus at different stages of gestation. The influence of genetic differences between mother and fetus on the cell migration was also addressed by studying various types of matings: syngeneic, allogeneic and outbred. The frequency of maternal-fetal cell exchanges within the placenta is much higher in syngeneic and allogeneic gestations than in outbred ones. Maternal cells were mainly localized in the labyrinth where they were scattered or sometimes grouped in or near blood spaces. Groups of maternal cells could also be observed in maternal blood sinuses of the spongiotrophoblast. Conversely, fetal cells were organized in rings surrounding maternal blood sinuses in the decidua at 10–12 days of gestation. After day 13, they invaded the decidua. Fetal cells could also be detected in maternal peripheral blood and organs by nested PCR and fluorescence microscopy on cryosections, respectively. This suggests a role in the establishment and maintenance of the maternal tolerance to the fetus.

Keywords: Placenta; Microchimerism; Transgenic mouse; EGFP

1. Introduction

Cell exchanges between mother and fetus during pregnancy are a well-demonstrated occurrence in humans, in particular from numerous studies designed to develop non-invasive prenatal diagnosis or to prevent maternal cell contamination in cord blood samples used for transplantation. Long-term persistence, even for decades, of low numbers of fetal or maternal cells, has been described in the mother or progeny [1,2], respectively. These phenomena called ''microchimerism'' could be playing a role in different physiological or pathological phenomena.

Fetal microchimerism could be involved in the maternal tolerance to the fetus. It has been shown that the mother's immune system is tolerized by fetal [3,4] and placental antigens [5]. Increased chimerism has been associated with autoimmune pathologies [6,7]. However, the contribution of fetal cells to the triggering of autoimmune diseases or the repair of damaged maternal tissues [8–11] remains to be shown. Conversely, microchimerism of maternal origin could be playing a role in the vertical transmission of infectious agents [12], or in autoimmune diseases [13,14].

Unfortunately, studies on human microchimerism are limited by the amount of tissue available, the difficulty of obtaining accurate pregnancy histories, and the ethical impossibility

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of collecting tissue samples from healthy individuals. The laboratory mouse is a very useful model as its placentation is hemochorial and comparable, although not identical, to that of humans [15]. Numerous studies on fetal-maternal cell exchanges in mice have provided new insights on microchimerism [11,16–25]. However, cell migrations between mother and fetus within the placenta have not been described up to now, even though the placenta is the main interface between the two organisms and is likely to be a pathway for migrating cells. We have taken advantage of Enhanced Green Fluorescent Protein (EGFP) transgenic (Tg) mouse models [26,27] already successfully used by us [16] and others [11,22,23,25] to study microchimerism- to describe the passage of fetal cells into the maternal decidua and reciprocally, the migration of maternal cells into the fetal part of the placenta. Indeed, these models are very useful to visualize the development of the maternal-fetal interface. We were also interested in the influence of fetal-maternal histocompatibility on the placental microchimerism and on the passage and persistence of fetal cells in maternal blood and organs. Our results suggest that cell passages either way are frequent and that at least some of them occur via the placenta.

2. Materials and methods

2.1. Mice

EGFP-Tg mice on C57Bl/6 background (B6, H-2^b) were provided by M. Okabe (Genome Information Research Center, Osaka, Japan) [26] and EGFP-Tg mice on ICR background (outbred) were given by A. Nagy (Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada) [27]. Non-Tg B6 (H-2^b) or FvB (H-2^q) females were crossed with EGFP/EGFP B6 males, whereas non-Tg ICR females were mated with EGFP/EGFP ICR males, to detect fetal Tg cells in the maternal body. Conversely, EGFP/+B6 females were crossed with non-Tg C57Bl/6 or FvB males and EGFP/+ ICR females with non-Tg ICR males. The non-Tg fetal-placental units were analyzed to detect Tg maternal cells.

Mice were bred and maintained at the J. Monod Institute's facilities. All animal care and handling were performed according to institutional guidelines. The day of the vaginal plug was considered as day 0 of gestation.

2.2. Organ and cell preparation

Pregnant female mice were anesthetized and bled by intra-cardiac puncture. Placenta, maternal thymus, spleen and para-aortic lymph nodes were harvested and rinsed several times in PBS. Organs were fixed in 4% paraformaldehyde in PBS for 1 h at 4 °C and incubated over night at 4 °C in 25% sucrose in PBS. They were embedded in Cryomatrix (Shandon) and quickly frozen in liquid nitrogen. Six-micron cryosections (Leica) were placed on Superfrost-plus slides (CML) and stored at −80 °C. Air-dried cryosections were stained for 3 min with Hoechst (0.2 μg/mL) and washed several times in PBS. The slides were mounted in fluorescence mounting medium (DakoCytomation) with coverslips, and examined under a fluorescence microscope (Leica) equipped with an ORCA-ER camera (Nikon) and connected to a computer equipped with Lucia v4.5 software. We have calculated that a field observed under 10× magnification corresponds to an area of 400 × 600 μm. Routinely, 30 fields were examined per section.

Heparinized blood was obtained from anesthetized females by intracardiac puncture. Erythrocytes were eliminated by osmotic shock in lysing buffer (0.17 M Tris and 8.3 g/L ammonium chloride, mixed 1/9 [v/v], pH 7.3). DNA was isolated from the cell suspension.

2.3. Detection of the EGFP transgene by Nested PCR

In order to detect the EGFP transgene, PCR analyses were performed in a final volume of 25 μL containing 100 ng of purified genomic DNA in PCR $MgCl_2$-free buffer (Promega), 1/5 of Cresol Red (60% sucrose, 1 mM Cresol Red), 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 1 μM of the following primers specific for the EGFP transgene: 5′ TGGTGAGCAAGGGCGAGGAG 3′ and 5′ TCAGGTAGTGGTTGTCGGGC 3′, 1 U of Taq DNA polymerase (Promega), under the following conditions: a 1-min denaturing step at 94 °C followed by 40 amplification cycles (1 min at 94 °C, 1.5 min at 66 °C, 1 min at 72 °C), and a final 10-min elongation step at 72 °C. Two microliters of the amplification products were then reamplified in PCR $MgCl_2$-free buffer, 2 mM $MgCl_2$, 1/5 Cresol Red, 0.2 mM of each dNTP, 1 μM of the following primers: 5′ TTCAAGGACGACGGCAACTA 3′ and 5′ ATGGGGGTGTTCT GCTGGTA 3′, 1 U of Taq DNA polymerase as follows: 1 min at 94 °C, 30 amplification cycles (1 min at 94 °C, 1.5 min at 62 °C, 1 min at 72 °C) and 10 min at 72 °C. The sensitivity of the assay was estimated by diluting EGFP cells into non-Tg cell suspensions at ratios ranging from 1/5 to $1/10^6$. EGFP cells were still reproducibly detectable when they represented $1/10^5$ cells, but not $1/(5 \times 10^5)$ or $1/10^6$ (data not shown).

2.4. Statistical analyses

Mean numbers of migrating maternal or fetal cells in the placenta were compared using the Student *t*-test.

3. Results

3.1. EGFP-Tg mouse model

In a previous study [16], we have followed the migration of maternal cells into the offspring using an EGFP-Tg mouse model [26,27]. In the present work, we have used the same mice to describe the bi-directional cell trafficking between mother and fetuses within the placenta. To follow EGFP-Tg maternal cells into the fetal part of the placenta, EGFP-Tg/+ females were mated with non-Tg males. Conversely, non-Tg females were bred with EGFP-Tg/EGFP-Tg males, to detect fetal cells in the decidua or maternal peripheral blood and organs. Three different crossings were analyzed: syngeneic matings within a single inbred strain (C57/Bl6 background × C57/Bl6 background); allogeneic matings between two distinct inbred strains (C57/Bl6 background × FvB background); and outbred matings (ICR background × ICR background). This enabled us to study the effect of histocompatibility differences between mothers and fetuses on their reciprocal cell exchanges. It is important to note that in allogeneic crosses, all fetuses are different from the mother but identical to each other, whereas in outbred gestations, all fetuses are different from the mother and different from each other.

Implantation sites or placentas were harvested between days 6 and 19 *post-coïtum* (*dpc*), cryosectioned, and analyzed after Hoechst staining. We did not observe any cell exchanges between mother and fetuses before 10 *dpc*, but our model proved to be very useful to visualize the first stages following implantation and the development of the maternal-fetal interface. The precise juxtaposition between maternal and fetal tissues can be easily studied using the EGFP transgene expression in different combinations where it is inherited or not by the mother or the offspring.