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Natural history of fetal cell microchimerism during and following murine pregnancy

Kiarash Khosrotehrani, Kirby L. Johnson, Sarah Guégan, Helene Stroh, Diana W. Bianchi *

Division of Genetics, Departments of Obstetrics and Gynecology and Pediatrics, Tufts-New England Medical Center, 750 Washington Street, P.O. Box 394, Boston, MA 02111, USA

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Abstract

In humans, fetal cells enter the maternal circulation during all pregnancies and can persist for decades. Human studies, however, are often limited by the number of subjects and the availability of healthy and diseased tissues for analysis. We sought to develop a murine model to establish the natural history of fetal cell microchimerism in various maternal tissues during and after healthy pregnancies resulting from congenic and allogenic matings. We bred C57BL/6J and DBA/2J virgin female mice to C57BL/6J males transgenic for the enhanced green fluorescent protein (GFP), which shows autosomal dominant inheritance with complete penetrance and is under the control of a ubiquitous chicken beta-actin promoter and a cytomegalovirus enhancer. During pregnancy and at different times after delivery, female mice were sacrificed. Tissues were collected and the presence of the *gfp* transgene and GFP+ cells was assessed by real-time quantitative PCR and by immunofluorescence. During pregnancy, microchimerism was detected in all tissues from mice carrying GFP+ fetuses. Fetal cells were often mononuclear. The frequency of fetal cells in the lungs was significantly higher compared to other tissues. The level of microchimerism was also significantly higher in congenic compared to allogenic matings. After delivery, the frequency of fetal cells decreased and fetal cells were undetectable at 2 and 3 weeks after the first delivery. However, some mice that had three gestations had detectable fetal cells 3 weeks after their last delivery. Using sensitive methods of detection, we demonstrate that fetal cell microchimerism occurs during all murine pregnancies. We describe a useful model for the study of the consequences of this phenomenon.

Keywords: Fetal cells; Microchimerism; Tissues

* Corresponding author. Tel.: +1 617 636 1468; fax: +1 617 636 1469.
E-mail address: dbianchi@tufts-nemc.org (D.W. Bianchi).

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1. Introduction

During human pregnancy, fetal cells enter the maternal circulation (Ariga et al., 2001) and can persist in the maternal blood and tissues for decades (Bianchi et al., 1996). This phenomenon, defined as a stable state of apparent engraftment of allogenic fetal cells in the maternal body, is known as fetal cell microchimerism (Liégeois et al., 1977, 1981). Fetal cells are very rare in the maternal circulation and have been estimated to circulate at a frequency of 2–6 cells/mL of maternal blood (Krabchi et al., 2001). This level increases after termination of pregnancy (Bianchi et al., 2001), or in the setting of certain fetal or maternal abnormalities (Ganshirt et al., 1995; Bianchi et al., 1997; Holzgreve et al., 1998).

Human studies are often limited by the number of subjects and the availability of healthy and diseased tissues for analysis. To better study fetal cell microchimerism, animal models have been developed. The presence of fetal cell microchimerism during and after mouse pregnancy is well known (Liégeois et al., 1981). Using murine models, investigators have demonstrated the role of the maternal immune system in the engraftment of fetal cells during pregnancy (Bonney and Matzinger, 1997). However, most of the previous studies focused on hematopoietic tissues, although our group and others have demonstrated the presence of fetal microchimeric cells in non-hematopoietic tissues (Khosrotehrani and Bianchi, 2003). Using mice transgenic for reporter genes, one can easily distinguish fetal from maternal cells (Imaizumi et al., 2002). In this study, we bred congenic or allogenic wild-type females to males transgenic for the enhanced green fluorescent protein sequence. Fetal cells from pups that inherit the transgene fluoresce green and are easily detectable in maternal tissues. Using this model, we sought to establish the natural history of the transfer of fetal cells in various maternal tissues during and after pregnancy in healthy animals resulting from congenic and allogenic matings.

2. Materials and methods

2.1. Mice

The Institutional Animal Care and Use Committee (IACUC) of the Tufts University School of Medicine Division of Laboratory Animal Medicine approved the protocol described here. The enhanced green fluorescent protein transgenic mouse (GFP+) (Jackson Laboratories stock #03291, Bar Harbor, ME) has a C57BL/6J (H-2^b) genetic background with the enhanced green fluorescent protein (*gfp*) transgene which shows autosomal dominant inheritance with complete penetrance and is under the control of a ubiquitous chicken beta-actin promoter and a cytomegalovirus enhancer (Okabe et al., 1997).

We bred 8-week-old C57BL/6J (H-2^b) and DBA/2J (H-2^d) virgin female mice to C57BL/6J (H-2^b) GFP+ males. Mice were sacrificed at weekly intervals during pregnancy or one, two or three weeks after a first, second or third delivery. Female mice that did not deliver a litter after mating with a GFP+ male were excluded, to avoid potential confounding variables such as spontaneous abortion or resorption. After delivery, for each mouse we recorded the total number of pups and the number of GFP+ pups (based on observed green fluorescence under UV light). We also purchased C57BL/6J female retired breeders from