In this issue of Circulation Research, Kara et al present an elegant series of experiments demonstrating that after murine maternal myocardial infarction, fetal cells engraft and differentiate into endothelial cells, smooth muscle cells, and cardiomyocytes at the site of injury. These cells, when isolated and cocultured with neonatal cells, appear to be capable of differentiating into beating cardiomyocytes. These results, if validated by other investigators, have profound significance with regard to gender biology. Although it has been known for more than a century that as a result of pregnancy women naturally transiently acquire a population of cells from their fetuses, more recent studies have shown that the fetal cells actually persist in maternal blood and organs for decades after delivery. Although initially the focus was on the association between fetal cells and the increased incidence of autoimmune diseases in women, later investigators used animal models to explore the potential for the pregnancy-associated progenitor cells to differentiate and repair injured or diseased maternal organs. For example, Nguyen Huu et al showed that fetal cells preferentially migrated to the injured skin in a murine model of contact dermatitis induced during pregnancy. Pregnancy-associated progenitor cells also were shown to cross the murine blood–brain barrier and integrate within the maternal brain in a model of Parkinson disease. The fetal cells in maternal brain had morphologies similar to neurons and expressed neuron-specific genes such as NeuN and β3-tubulin. Together, these findings support the hypothesis that fetal cells cross the placenta as progenitor cells and differentiate within the maternal tissues.

In the Kara et al study, female B6CBA mice were mated with C57B16 males that carried one copy of egfp, allowing detection of cells that expressed green fluorescent protein (GFP) from the 50% of pups that inherited the transgene. The left anterior descending coronary artery was ligated during pregnancy (day e12) and the maternal hearts were studied at 1 to 4 weeks after injury. Four different types of experiments were performed: (1) quantitation of the number of fetal cells in infarcted, control, and sham exposure hearts; (2) dual color immunofluorescence studies examining differentiation mark-

ers in the GFP+ cells; (3) fluorescence in situ hybridization using X and Y chromosome probes to rule out fusion between male fetal and female maternal cells; and (4) isolation and clonal expansion of the GFP+ cells.

The study team demonstrated, using real-time quantitative polymerase chain reaction techniques, that there was more GFP expression in infarcted hearts than in controls. Yet, sham surgery also resulted in increased trafficking of fetal cells to the heart compared to controls. That there were differences observed between both sham and infarcted hearts versus controls suggests that fetal cells are mobilized in the presence of injury and inflammation. We have previously shown that chemical injury (exposure to CCl4), but not surgical injury (partial hepatectomy), results in an increased number of fetal cells in postpartum murine maternal liver. We hypothesized that our results were attributable to the different mechanisms required for liver repair after the two types of injuries: repair after CCl4 exposure relies on bone marrow stem cells in addition to hepatic oval cells, whereas regeneration after partial hepatectomy mainly involves hepatocyte division. In future experiments it will be important to test whether inflammation is in fact necessary for the increased presence of fetal cells at sites of maternal injury.

In the current study, the authors stated that they showed “selective and specific homing” to the maternal heart. In fact, an alternate explanation of their results is that the fetal cells proliferated in situ in response to the myocardial infarction. Furthermore, it is significant that the injury was performed at day e12. Fetomaternal cell trafficking in the mouse is detectable after day e10 and peaks immediately before delivery at days e18 and e19. Thus, the cardiac injury occurred at a time when fetal cells were physiologically circulating in the mother. In future experiments, it will be critical to determine if similar results are achieved if the injury is performed after delivery. If this occurs, then it will broaden the significance of these findings beyond peripartum cardiomyopathy. It is also essential to determine whether fetal cells in the maternal heart result in any significant functional improvement after myocardial injury. The authors did not perform any functional evaluation before euthanasia. A logical next step would be to compare the extent of recovery from myocardial infarction in pregnant, postpartum, and virgin females.

One of the most important questions today within the field of fetal cell microchimerism is, what are the specific types of fetal cells that are present in the maternal organs? Although some researchers have suggested that fetal cells may have a uniform phenotype somewhere between adult and embryonic stem cells, others have demonstrated that they represent a mixed population. Some fetal cells express stem cell markers such as CD34, CD44, and CD184/CXCR4, and others ex-

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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press antigens suggestive of more mature phenotypes, such as CD45 (a leukocyte marker) or CD105 and CD31, which are found on endothelial cells.

One of the strengths of the study by Kara et al is that they examined a number of embryonic stem cell markers (Sox2, Nanog, and Pou5f1), as well as markers expressed by cells differentiating along the cardiac lineage (Sca-1, c-Kit, Islet1, and Nkx2.5). Forty-six percent of the cells expressed CD31, which is consistent with previous studies that indicate that the fetal cells participate in angiogenesis. This study demonstrated the novel finding that 40% of fetal cells isolated from the maternal heart express Cdx2, the first of three causal homeobox genes to be expressed during development. Cdx2 is responsible for identifying the cells destined to become the trophectoderm from those that will form the inner cell mass. Cdx2 is expressed in trophoblast stem cells but is absent in the mature trophoblast. Because of these facts, the authors suggest that the cells with regenerative potential may have originated from the placenta. Although trophoblasts are known to travel to the human lung during pregnancy, trafficking of trophoblasts in murine pregnancies has not been previously reported. Further support for a placental origin of the GFP+ cells comes from a study in which mesenchymal stem cells isolated from human amniotic membranes were tolerated by a murine host and differentiated into cardiomyocytes 2 weeks after induced myocardial infarction.

In light of these reports, it is tempting to speculate that the placenta contains a reservoir of stem cells with the potential to differentiate into beating cardiomyocytes. However, Cdx2 is not exclusively expressed in trophectoderm. Along with Cdx1, Cdx2 is expressed in the intestinal hindgut epithelium in the embryo; expression persists throughout life in the distal colon. Additionally, trophoblasts are cleared from the maternal circulation almost immediately after parturition. Thus, the cells with regenerative potential may not be trophoblasts. Alternate explanations for the results include a different population of stem cells from the fetus that express Cdx2 or another placental cell type. In any case, it is the mother who benefits from the cells with the regenerative potential.

A remarkable aspect of the Kara et al study was that they induced the isolated fetal cardiomyocytes to beat in vitro when cultured on a neonatal feeder layer. Fusion as an explanation for these results was excluded by fluorescence in situ hybridization studies. Similar results were achieved in another study when amniotic membrane-derived mesenchymal stem cells transdifferentiated into cardiomyocytes. Separation of the isolated fetal cells and the feeder layer by a membrane permeable only by small molecules may eliminate fusion as a possibility, while still permitting cell-to-cell communication.

Two important conclusions can be derived from this study. One is that after myocardial infarction, fetal cells that express both embryonic and more differentiated markers can be preferentially found at the site of injury. The other is that the placenta, which is normally discarded in the human after delivery, may be a valuable source of cells with regenerative potential. Fetal cell microchimerism has been repeatedly demonstrated in rodent models, primates, and humans. A number of reports have now shown in multiple species that fetal cells have the capacity to express differentiation markers consistent with the adult tissue in which they are found. The fact that female adult organs contain progenitor or stem cells from their fetuses would seem to be a major biological difference between women and men, yet the mainstream stem cell community has not paid much attention to this fact. The advances presented here by Kara et al will hopefully re-awaken scientific and medical interest in further exploration of the functional significance of and underlying mechanisms associated with fetal cell microchimerism. On a more human level, it is fascinating to realize that after the mother spends 9 months providing nutrients and an environment for optimal growth and development of the baby, the baby gives back cells with regenerative potential to the mother.

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Disclosures
None.

References


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