Multi-lineage potential of fetal cells in maternal tissue: a legacy in reverse

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Summary

Fetal cells circulate in pregnant women and persist in blood and tissue for decades post-partum. The mother thus becomes chimeric. Factors that may influence such fetal cell microchimerism include histocompatibility, fetal or placental abnormalities, or a reproductive history that includes miscarriage or elective termination. Fetal cell microchimerism is associated with some maternal autoimmune diseases, such as systemic sclerosis. Moreover, a novel population of fetal cells, the pregnancy-associated progenitor cells (PAPCs), appears to differentiate in diseased or injured maternal tissue. The cellular origin of these cells is at present unknown but could be a hematopoietic stem cell, a mesenchymal stem cell, or a novel cell type. Pregnancy therefore results in the acquisition of cells with stem-cell-like properties that may influence maternal health post-partum. Rather than triggering disease, these cells may instead combat it.

Key words: Stem cells, Pregnancy, Fetus, Fetal cell microchimerism, Pregnancy-associated progenitor cells

Introduction

The recent discovery of the long-term persistence of fetal cells in maternal blood and tissues decades after pregnancy has opened up a new field of investigation (Bianchi et al., 1996; Nelson et al., 1998; O'Donoghue et al., 2004). Fetal cell microchimerism, originally described in mice, is defined as the persistence of fetal cells in maternal organs and circulation without any apparent graft-versus-host reaction or graft rejection (Liégeois et al., 1977). It has become apparent that fetal cell microchimerism is a widespread phenomenon, and we now know that fetal cells are transferred to the maternal circulation during all human pregnancies and after delivery. The findings in humans have led to the hypothesis that autoimmune diseases that are found predominantly in women may result from an immune reaction between the mother and the fetal cells that remain post-partum (Nelson, 1996). Persisting fetal cells are also found in a wide range of tissues from women affected with a variety of non-immune diseases, such as hepatitis C and cervical cancer.

Here we discuss recent findings that suggest that, in all pregnancies, fetal cells that have stem-cell-like properties are transferred into maternal blood. We hypothesize that these cells, which we term pregnancy-associated progenitor cells (PAPCs), persist after delivery in a maternal stem cell niche and, in the case of tissue injury, home to the damaged organ and differentiate as part of the maternal repair response.

Fetal cells circulate during pregnancy

In all human pregnancies, fetal cells can be detected in the maternal circulation (Ariga et al., 2001). Feto-maternal cell trafficking starts as early as six weeks of gestation (Ariga et al., 2001). The frequency with which fetal cells can be detected in blood from pregnant women increases with gestational age. In normal second-trimester pregnancies, the number of fetal cells in the maternal circulation is estimated to be 1-6 cells/mL of maternal venous blood (Bianchi et al., 2001; Krabchi et al., 2001; Bianchi et al., 1997). At 36 weeks of gestation, 100% of pregnant women have fetal cells in their circulation (Ariga et al., 2001). After delivery, this fraction rapidly decreases. Sensitive PCR techniques indicate that 30-50% of healthy women have detectable fetal cells in their blood from four weeks to decades after delivery (Ariga et al., 2001; Artlett et al., 2002; Lambert et al., 2002). By examining specific peripheral blood mononuclear cell sub-populations, one can detect microchimerism in as many as 90% of healthy post-partum women (Evans et al., 1999). Fetal cell microchimerism is thus probably a widespread phenomenon, although difficult to detect.

Factors that influence the transfer of fetal cells during and after pregnancy

The amount of fetal cell transfer to the maternal circulation during pregnancy may be influenced by feto-maternal histocompatibility. In animal models, female mice with a syngenic fetus (one with identical histocompatibility alleles at the *H-2* locus) have higher numbers of microchimeric cells in their hematopoietic tissues, such as blood, lymph node and spleen, compared with female mice with allogenic fetuses (those that have different histocompatibility alleles at the *H-2* locus) (Bonney and Matzinger, 1997). However, in humans, the same trend is not observed between feto-maternal histocompatibility and the persistence of fetal cell

microchimerism (Evans et al., 1999). Although certain maternal HLA alleles, such as HLA-DQ A1*0501, appear to be more frequently associated with fetal cell microchimerism (Lambert et al., 2000; Nelson et al., 1998), this finding is controversial (Artlett et al., 2003).

The number of fetal cells in the maternal circulation is affected by fetal and placental abnormalities. There is increased fetomaternal cell transfer in cases of fetal aneuploidy (Bianchi et al., 1997), maternal preeclampsia (Holzgreve et al., 1998) or following terminations of pregnancy (Bianchi et al., 2001). In the latter case, in the second trimester, the number of fetal cells in the maternal circulation before and after a termination increases from 19 to 1500/16 mL of maternal whole blood.

A woman's reproductive history is also important. By systematically analyzing all published cases of microchimerism that described the study subjects' individual reproductive histories, we observed that a prior history of fetal loss (either miscarriage or termination) significantly increases the chance that fetal cells can be detected in that woman's organs (Khosrotehrani et al., 2003a). Women who have a history of fetal loss are 2.4 times more likely to exhibit fetal cell microchimerism than are women with no history of fetal loss. Unfortunately, our meta-analysis cannot distinguish between natural and voluntary pregnancy loss in the published literature. There may be significant differences in the incidence of microchimerism between these scenarios. Other variables, such as the number of pregnancies, do not appear to influence the persistence of fetal cells significantly. The increased microchimerism following fetal loss is probably due either to increased transfusion of fetal cells at the time of loss or to the transfer of a cell type that is at an earlier developmental stage and thus more likely to engraft in the mother.

Another factor that might influence the presence of microchimerism is the length of time that has elapsed since completion of pregnancy. Several studies have suggested that fetal cells are not detectable in women with younger sons (Bianchi et al., 1996; Filho et al., 2002).

Fetal stem cells are transferred during pregnancy from the fetus to the mother

In all human pregnancies, fetal progenitor cells that express CD34 are transferred into the maternal circulation (Guetta et al., 2003); they can be isolated by culturing maternal blood during pregnancy and up to six months after delivery (Osada et al., 2001). The number of fetal progenitor cells circulating in the blood of pregnant women has been estimated to be 0-2/mL (Guetta et al., 2003). Decades after delivery, male fetal $CD34^+$ (hematopoietic stem cells; HSCs) and $CD34^+CD38^+$ cells (which are committed to early B- and T-cell development) have been identified in 75% of women with sons (Bianchi et al., 1996). In addition, fetal cells have been detected in the $CD34^+$-enriched fraction of apheresis products after growth-factor-induced mobilization of HSCs in 50% of the women studied (Adams et al., 2003). (Apheresis is a procedure in which blood is drawn and separated into its components by dialysis; $CD34^+$ cells are retained and the rest are returned to the donor.)

During the first trimester of pregnancy, fetal blood also contains mesenchymal stem cells (MSCs) (Campagnoli et al., 2001), which were initially described in adult bone marrow. Microchimeric fetal MSCs have been isolated from the peripheral blood of an adult woman following termination of pregnancy (O'Donoghue et al., 2003). Fetal stem cells thus seem to enter the maternal circulation during pregnancy and persist in niches such as bone marrow.

Do fetal cells cause autoimmune disease?

The long-term presence of fetal cells in the semi-allogenic maternal body raises the possibility of an immune reaction between maternal and fetal cells that results in maternal disease. Nelson has hypothesized that some autoimmune diseases that preferentially occur in middle-aged women, and that have clinical and pathological similarities with graft-versus-host reaction disease, may in fact be allo-immune diseases (Table 1) (Nelson, 1996). Systemic sclerosis (SS) is such a disease. The number of fetal cells present in blood and other tissues of women affected with SS is significantly higher than in controls (Artlett et al., 1998; Nelson et al., 1998; Khosrotehrani and Bianchi, 2003). Johnson et al. studied autopsy specimens from multiple tissues from women affected with SS and showed that male cells of putative fetal origin were most frequently observed in spleen (Johnson et al., 2001a). This group also reported that, in a woman with systemic lupus erythematosus (SLE) who died of severe intestinal vasculitis, affected tissues had more fetal cells present than did healthy tissues (Johnson et al., 2001b). Similar techniques do not reveal any microchimeric fetal cells in skin samples from a group of

Table 1. The association between fetal cell microchimerism and maternal autoimmune diseases

Disease	Tissues studied	Techniques used to detect microchimerism	Incidence of microchimerism compared with controls
Systemic sclerosis	Peripheral blood	Y chromosome or HLA PCR	Significantly increased
	Affected tissues	Y chromosome PCR and FISH	Significantly increased
Polymorphic eruption of pregnancy	Skin	Y chromosome PCR	Significantly increased
Systemic lupus erythematosus	Many	Y chromosome PCR and FISH	Variable according to disease severity
Primary biliary cirrhosis	Liver	Y chromosome PCR and FISH	No significant differences*
Sjögren's syndrome	Salivary glands and peripheral blood	Y chromosome PCR	No significant differences†
Hashimoto's disease	Thyroid	Y chromosome PCR or FISH	Significantly increased
Graves' disease	Thyroid	Y chromosome PCR	No significant differences
Cutaneous lichen planus	Skin	Y chromosome PCR	No significant differences

*When primary biliary cirrhosis presents in association with systemic sclerosis, or diseases related to systemic sclerosis, significantly increased microchimerism has been found (Corpechot et al., 2000).

†When Sjögren's syndrome presents in association with systemic sclerosis, significantly increased microchimerism has been found (Aractingi et al., 2002).

women affected with mild SLE (Khosrotehrani et al., 2005a). Filho et al. detected evidence of Y-chromosome sequences in the blood of 68% of SLE patients compared with 33% in controls (Filho et al., 2002). Patient disease severity was not described in this study. In SLE, fetal cell microchimerism might be more likely to be found in affected tissues of severe cases (for example with nephritis) rather than benign ones (Mosca et al., 2003). Fetal cells thus probably do not trigger the disease but instead home to the affected maternal tissue if the damage reaches a particular 'threshold'.

Does the fetus 'treat' its mother?

What if the fetal cells are found in the clinically affected organs because they are attempting to combat the disease? We have analyzed a liver biopsy specimen from a woman with hepatitis C (Johnson et al., 2002) who stopped treatment (against medical advice) but, despite this, did well clinically and her disease abated. Her liver specimen contained thousands of male cells detected by dual-color fluorescence in situ hybridization (FISH) studies using probes for the X and Y chromosomes. She had never received a blood transfusion and was not a twin. Follow-up studies using DNA polymorphism analyses indicated that the probable source of the male cells in her liver was a pregnancy that she had terminated 17-19 years earlier (Johnson et al., 2002). In this case, the male cells in the liver were morphologically indistinguishable from surrounding liver tissue, which suggests that they were hepatocytes.

Similarly, a study of biopsy material from 29 women with thyroid disorders revealed fetal microchimeric cells in women with Hashimoto's disease as well as other non-immune thyroid disorders (Srivatsa et al., 2001). An unexpected result was the detection of large numbers of male fetal cells in an otherwise healthy woman who had a benign thyroid adenoma. DNA probes that map to the X and Y chromosomes showed that mature follicles from the woman's thyroid were partly male and partly female. She had no other potential sources of microchimeric cells: she had never been transfused, had never had an organ transplant, and was not a twin.

More systematic studies have examined the phenotypes of fetal microchimeric cells from patients who have high numbers of such cells by combining in situ hybridization to detect the fetal cells and immunolabeling to identify their phenotype (Khosrotehrani et al., 2003b). In epithelial tissues such as thyroid, cervix, gallbladder or intestine, 14-60% of the fetal cells express epithelial markers such as cytokeratin (Fig. 1). In the liver, 4% of the fetal microchimeric cells have a hepatocytic phenotype (Khosrotehrani et al., 2004a). Most of the other fetal microchimeric cells in these tissues express CD45, the common leukocyte antigen, indicating a likely hematopoietic origin. Similarly, 90% of the fetal cells detected in maternal hematopoietic tissues, such as spleen or lymph node, express CD45. In all cases, the morphology of the fetal cells suggests that they have differentiated. In addition, in sections containing diseased and healthy thyroid tissue, the fetal cells more frequently express cytokeratin if they are in the diseased area of the thyroid. These results suggest that fetal cells, possibly hematopoietic in origin, home to the site of injury and adopt the maternal local tissue phenotype. Whether the fetal cells actually differentiate or fuse with the damaged host cells remains an open question. However, using chromosome-specific probes and FISH analysis, we have never observed the tetraploid signals that would be consistent with such fusion. We therefore conclude that, among the fetal cells transferred to the mother during pregnancy, some have multi-lineage capacity. We term these PAPCs.

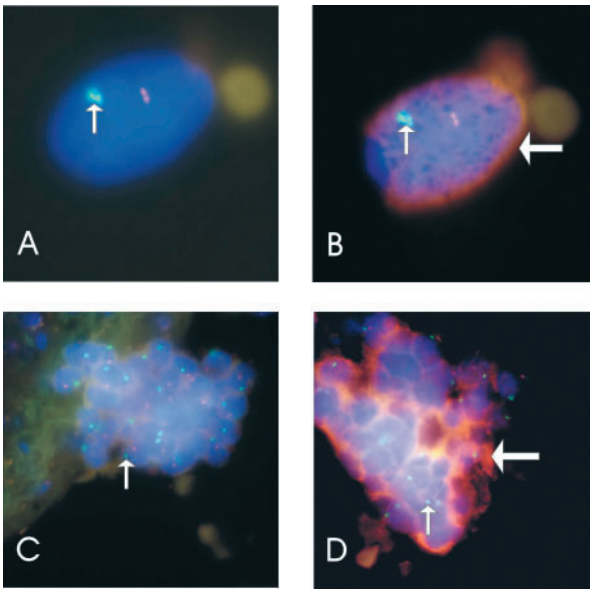

Fig. 1. Microchimeric fetal cells in female thyroid express cytokeratin. Photomicrographs show FISH studies using Cy3-labeled X (orange) and FITC-labeled Y (green) chromosome probes, and immunofluorescence staining with anti-cytokeratin antibody, Texas Red (red). Nuclei are counterstained with DAPI (blue). (A) Male microchimeric cell in maternal thyroid with one Y chromosome (small arrow) and one X chromosome; 1000× magnification. (B) Same cell stained with anti-cytokeratin antibody (large arrow, red), indicating an epithelial cell; 1000× magnification. (C) Interphase FISH study of female thyroid tissue showing a group of male microchimeric cells; 400× magnification. Arrow shows one X and one Y chromosome. This group of cells did not stain positively for cytokeratin; therefore, they are not epithelial cells. (D) Combined FISH and immunofluorescence staining of a group of male microchimeric cells with one X and one Y (small arrow) chromosome. Note that in this plane of focus not all of the X chromosomes can be seen. This group of cells expresses cytokeratin (large arrow); 400× magnification.

Cellular origin of the PAPCs

Fetal $CD34^+$ and $CD34^+CD38^+$ cells circulate in maternal blood for decades after delivery (Bianchi et al., 1996). We believe that, to persist long-term, the fetal microchimeric cell population must contain stem cells that can proliferate, as initially proposed for bone marrow cell microchimerism (Liégeois et al., 1977). It is hard to imagine how fully differentiated fetal cells that have a short half-life and no self-renewal capacity could regularly appear in maternal blood and tissue decades after delivery. Thus, we hypothesize that they have stem-cell-like properties. Both fetal blood and the placenta contain various types of stem cells. HSCs have been identified, isolated and cultured from the placenta. In fact, the placenta contains 2-4 times more HSCs than other fetal hematopoietic tissues, such as the liver or yolk sac (Alvarez-

Silva et al., 2003). In addition, placental HSCs have a higher proliferation potential than fetal liver progenitors, which are at a later developmental stage.

Another possible origin of the PAPCs could be MSCs. Recently, O'Donoghue et al. found male (presumed fetal) MSCs in 100% of bone marrow samples obtained at thoracotomy from women with sons who ranged in age from 13 to 51 years (O'Donoghue et al., 2004). They characterized these cells phenotypically, as well as functionally, following culture. Under appropriate culture conditions, the cells differentiate into muscle, nerve, bone and fat.

The need for animal models

Studies of human fetal microchimerism are limited by the amount of appropriate tissue available, the difficulty of obtaining accurate pregnancy histories, and the impossibility of analyzing tissue from healthy individuals. Recently, several animal models have been used in studies of fetal cell microchimerism. Jimenez and Tarantal used rhesus monkeys (*Macaca mulatta*) to study feto-maternal trafficking (Jimenez and Tarantal, 2003) and demonstrated long-term persistence of male $CD34^+$ cells in one or more maternal tissues (Jimenez et al., 2005). Fetal cell microchimerism has also been examined in mice (Liégeois et al., 1981; Bonney and Matzinger, 1997; Christner et al., 2000) and, more recently, in rats (Wang et al., 2004).

Most studies of murine fetal cell microchimerism rely on the fetus and the mother being of different sex, or on the presence of a marker chromosome (T6) in the fetus. More recently, we have used male transgenic mice carrying unique paternal reporter transgenes to identify and track the fetal cells. The transgenic fetal cells can be easily detected in the wild-type maternal tissues. For example, when enhanced green fluorescent protein (GFP) under the control of the chicken β-actin promoter and the cytomegalovirus (CMV) enhancer is used as the reporter (Okabe et al., 1997), cells from transgenic fetuses can be easily detected in wild-type females by fluorescence microscopy or immunohistochemistry. Furthermore, quantifying the number of *gfp* sequences by real-time PCR amplification of genomic DNA allows detection of the equivalent of one fetal cell in 10^5 maternal cells (Khosrotehrani et al., 2004b). These methods allow fetal cells to be monitored in maternal blood and tissue during and after normal murine pregnancies (Khosrotehrani et al., 2005b). In addition, we are currently developing injury models to assess the capabilities of fetal cells to home to maternal injured tissues and to differentiate. Animal models show great promise for determination of which types of maternal injury or disease are most likely to recruit fetal cells from their niche. Furthermore, new bioluminescent imaging techniques will allow study of the behavior of these cells in the living mouse (Contag et al., 1998).

Conclusions/Perspectives

Fetal cell microchimerism is a new field of investigation. It is a widespread phenomenon that potentially affects every woman who has been pregnant. The discovery of the long-term persistence of fetal cells in maternal tissues, with their evidence of multi-lineage capacity, strongly suggests the presence of a novel population of cells that are acquired physiologically. The work discussed here provides strong evidence that all adult stem cells are not alike. In the future, consideration must be given to whether adult stem cells originate from a male or female and whether that female has been pregnant, since tissues from adult parous females appear to contain a mixed population of adult and fetal cells.

Pregnancy results in the acquisition of cells that may have clinical applications and therapeutic potential. Whether the PAPCs are HSCs or MSCs, or a new population of stem cells, is an unresolved issue. It is also unknown whether PAPCs respond to all types of maternal injury or only those injuries that recruit stem cells. It is possible that these cells, since they are fetal in origin, have a higher proliferative capacity or more plasticity than their equivalent adult (maternal) cells. In the current debate over the use of embryonic stem cells for treatment of disease, the discovery of a population of fetal stem cells that apparently differentiate in the adult woman and can be acquired without harming the fetus may be significant. Future research will focus on animal models to determine the contribution of the fetal PAPCs to the repair of maternal injury.

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