Forever connected: the lifelong biological consequences of fetomaternal and maternofetal microchimerism

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Clinical Chemistry 67:2
351–362 (2021)

Review

Forever Connected: The Lifelong Biological Consequences of Fetomaternal and Maternofetal Microchimerism

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BACKGROUND: Originally studied as a mechanism to understand eclampsia-related deaths during pregnancy, fetal cells in maternal blood have more recently garnered attention as a noninvasive source of fetal material for prenatal testing. In the 21st century, however, intact fetal cells have been largely supplanted by circulating cell-free placental DNA for aneuploidy screening. Instead, interest has pivoted to the ways in which fetal cells influence maternal biology. In parallel, an increasing appreciation of the consequences of maternal cells in the developing fetus has occurred.

CONTENT: In this review, we highlight the potential clinical applications and functional consequences of the bidirectional trafficking of intact cells between a pregnant woman and her fetus. Fetal cells play a potential role in the pathogenesis of maternal disease and tissue repair. Maternal cells play an essential role in educating the fetal immune system and as a factor in transplant acceptance. Naturally occurring maternal microchimerism is also being explored as a source of hematopoietic stem cells for transplant in fetal hematopoietic disorders.

SUMMARY: Future investigations in humans need to include complete pregnancy histories to understand maternal health and transplant success or failure. Animal models are useful to understand the mechanisms underlying fetal wound healing and/or repair associated with maternal injury and inflammation. The lifelong consequences of the exchange of cells between a mother and her child are profound and have many applications in development, health, and disease. This intricate exchange of genetically foreign cells creates a permanent connection that contributes to the survival of both individuals.

For well over a century it has been known in the medical literature that multi-nucleated syncytial giant cells could be demonstrated in the organs of women who died of eclampsia (1, 2). The renowned German pathologist, Christian Georg Schmorl, unexpectedly and consistently found them when performing autopsies on 17 women. While Schmorl's focus was on understanding the pathophysiology of preeclampsia and eclampsia, his work established the foundation for a body of knowledge that has continuously evolved over time. In this review, we will discuss the potential clinical applications and functional consequences of bi-directional trafficking of intact cells between the pregnant woman and her fetus. Originally sought as a source of fetal material that could be noninvasively obtained for prenatal genetic diagnosis, the information presented here will highlight the potential role that fetal cells play in the pathogenesis of maternal disease and tissue repair, as well as the importance of maternal cells in educating the fetal immune system. This intricate interchange of genetically foreign cells creates a lifelong bond between a mother and her child that contributes to the survival of both individuals.

Fetomaternal Microchimerism

WHERE FETAL CELLS ARE FOUND IN THE MOTHER

Maternal blood. Historically, the placenta was thought to be a barrier that separated the genetically distinct mother and fetus. It is now well established, however, that fetal cells pass into the maternal circulation during both human and rodent gestations (3, 4). Definitive proof of fetal cells in maternal blood occurred when XY metaphases in fetal lymphocytes were demonstrated in the peripheral blood of pregnant women carrying male fetuses (5). Even more convincing evidence came in the 1990s with the application of molecular genetic

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Received August 29, 2020; accepted October 28, 2020.
DOI: 10.1093/clinchem/hvaa304

techniques such as polymerase chain reaction amplification and fluorescence in situ hybridization to detect unique amplified fetal DNA sequences from cellular components of the blood of pregnant women (4).

Fetal cells can be found in the peripheral blood of 100% of pregnant women and are detectable by 6 weeks of gestation, although the volume of fetal blood present in the maternal circulation is usually very small (6). Data from a review of fetomaternal hemorrhage show that the volume present in the maternal circulation at delivery is < 0.05 mL in 74% of women, < 1 mL in 96%, and < 30 mL in 99.67% (7). The principal mechanism is damage to placental villi with physical disruptions in the 5–45 μm barrier that separates the maternal and fetal circulations (Fig. 1). The number of fetal cells found in the maternal circulation is influenced by fetal and placental pathologies. Fetal cells can pass into maternal blood in significant amounts during or after spontaneous miscarriage (8, 9). Fetomaternal hemorrhage after first trimester termination of pregnancy results in an 80-fold increase in fetal cells in maternal blood (10). An increased number of fetal cells is also observed in maternal blood in cases of fetal aneuploidy, although this may reflect placental abnormalities rather than the underlying karyotype (11). More recently, cesarean delivery has been conclusively linked with higher detection and greater concentrations of microchimeric cells in maternal blood than vaginal delivery (12). Fetomaternal cell trafficking is increased in a range of common pregnancy problems, including hyperemesis gravidarum, preeclampsia, antepartum hemorrhage, and miscarriage (13–19).

Estimates during typical pregnancies suggest there are only 1 to 2 fetal cells per mL of maternal blood (11), with trophoblasts, monocytes, B and T lymphocytes, nucleated erythrocytes, and hematopoietic progenitors present (Table 1). In practice, stimulating fetal erythroid and hematopoietic progenitor cells in culture to provide a greater number for genetic analysis proved disappointing, because fetal and maternal cells were too similar, and amplification was unsuccessful (20).

The identification of mesenchymal stem cells (MSC) in first trimester fetal blood (21) offered the prospect of targeting a unique fetal stem cell for noninvasive prenatal diagnosis. Fetal MSC were, however, found to circulate at a very low frequency in maternal blood (in 5% of post termination blood samples), making them unlikely to be useful clinically (22).

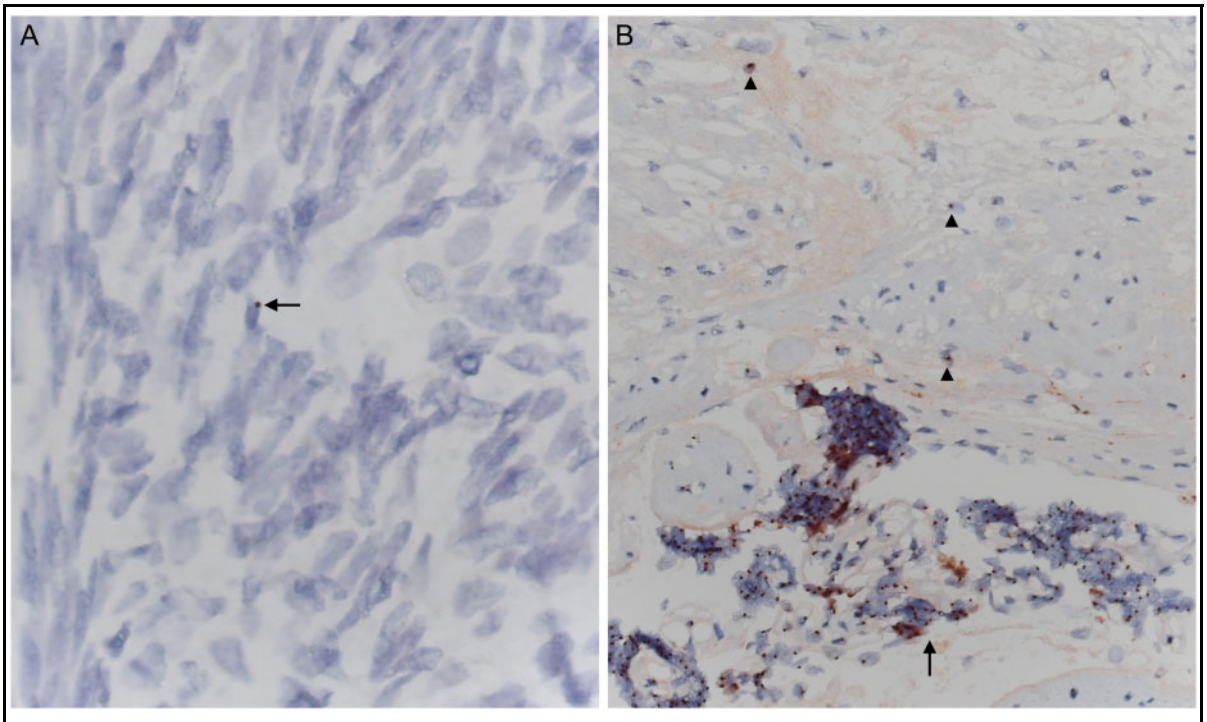

Fig. 1. Male fetal chimeric cells in the uterus identified by in situ hybridization. (A) A single Y-chromosome positive cell in a healthy control myometrium; (B) A uterus with placenta increta. Large numbers of chimeric cells are present at sites of placental invasion (arrow); arrowheads show individual male cells. Figure courtesy of Marije Koopmans and Idske Kremer Hovinga.

Table 1. Types of fetal cells found in maternal blood.

	Trophoblasts	Leukocytes	NRBCs	HSCs	MSCs	PAPCs
Specific cell markers	No, although HLA-G has potential	Yes	Yes, improving with use of embryonic hemoglobins	Yes	Yes, although defined by absence of markers	CD34+CD38+ Endothelial
Similarity to maternal cells	None	Yes	Yes, with exception of primitive fetal NRBCs	Yes, but have higher proliferative potential	None in blood	Not known
Found in non- pregnant adult circulation	No, cleared by pulmonary circulation	Yes	Yes	Not typically	No	No
Persist in maternal tissues	No	Yes	No, short-lived	Yes	Yes	Yes
Other qualities	Developmentally end-stage, Multinucleated	Identified by HLA differences between mother and fetus	Physiologic increase in maternal NRBCs occurs during pregnancy	Rare, but can be amplified in vitro	Rare, but can be amplified in vitro, may escape immune rejection. Likely to adhere and engraft rapidly	Have capability of differentiation to repair maternal tissues

Abbreviations: NRBCs, nucleated erythrocytes; HSCs, hematopoietic stem cells; MSCs, mesenchymal stem cells; PAPCs, pregnancy-associated progenitor cells; HLA, human leukocyte antigen.

Subsequent work found that fetal CD34+ cells could be detected in the maternal intervillous blood space from term placental chorion, suggesting that their point of origin resided in the fetal villi; these cells were not hematopoietic but had endothelial lineage characteristics (23). Failure to routinely isolate the stem/progenitor cells in maternal blood was eventually and variously attributed to their low frequency, to limitations of the sensitivity of enrichment methods, and/or to their engraftment in maternal tissues soon after their transplacental passage (19, 24, 25). More recently, however, researchers identified, isolated and cultured fetal microchimeric stem cells from a pluripotent maternal stem cell niche 24–26 years after the delivery of a 45-year old woman's sons (26). These cells expressed genes associated with a pluripotent stem cell phenotype and were able to be differentiated into osteocytes, adipocytes, and chondrocytes.

Maternal organs. Fetomaternal microchimerism is defined as low levels of intact fetal cells that persist in maternal blood and tissues for years after pregnancy (17, 19, 27, 28). Microchimerism is proposed as a state of balance between host versus graft and graft versus host reactions, leading to the acceptance of the allogenic fetus; it has been specifically suggested that fetal stem cells engraft in maternal bone marrow to maintain tolerance to the semi-allogeneic fetoplacental graft (29). Bianchi et al. (24) were the first to demonstrate that fetal cells could persist in maternal blood up to 27 years postpartum and later confirmed that a live birth was not required for a woman to become a chimera (10, 30, 31).

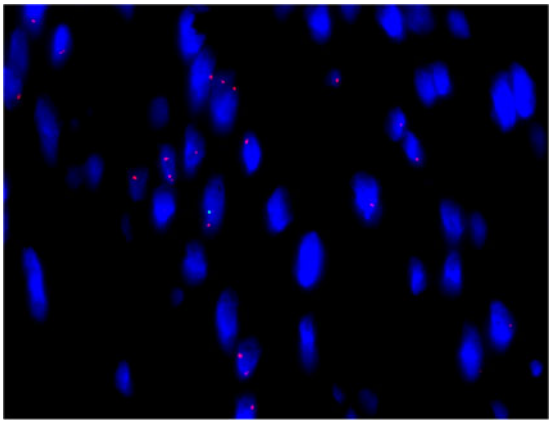

Fig. 2. Microchimeric male fetal cells present in the appendix of a pregnant woman. Fluorescence in situ hybridization was performed with X and Y chromosomes labeled with SpectrumOrange™ and SpectrumGreen™, respectively. A single male cell is seen in the center of the image.

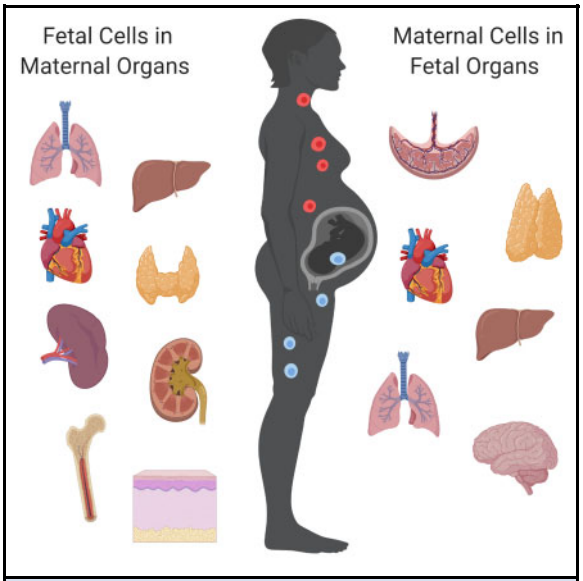

Fig. 3. Bidirectional trafficking of fetal and maternal cells during pregnancy. Fetal cells (red) become incorporated into maternal organs shown on the left. Maternal cells (blue) are found in fetal organs on the right. They play an important role in education of the fetal immune system.

To understand more about the significance and pathophysiological role of microchimeric cells, the 'where, when, and how' is of extreme importance. The search for microchimerism can make use of several sources, such as normal or diseased tissues; the timepoints can be during health or disease, during reproductive ages or beyond, during pregnancy or not. Techniques to detect chimeric cells in tissues usually rely on the detection of Y-chromosome positive cells in women (Fig. 2), sometimes in combination with immunohistochemistry to detect the true nature of the cells, but this technique limits the focus to women who have had male fetuses. Fetal cell microchimerism occurs equally in both fetal sexes. When studying chimeric cells in tissues, a distinction can be made between tissue samples obtained during life or at autopsy. The latter provides an adequate amount of tissue for study, but usually suffers from degenerative changes. Studies on the presence and location of chimeric cells in tissues from women obtained at autopsy have shown that Y-chromosome positive chimeric cells were present in kidneys, livers, hearts, thyroids, lungs, skins, and lymph nodes unaffected by disease (Fig. 3) (32, 33). It is evident that a low level of 'background' chimerism is physiologic and that the number of chimeric cells increases in disease. Whether the role of chimeric cells lies mainly in the development of disease or in its repair phase is still an unresolved issue (34).

Fetomaternal chimerism and maternal disease. Microchimerism seems to be more common in affected tissues than in blood (35), in which the number of microchimeric cells is greater than in controls (31). Studies have confirmed that the increase in fetal microchimerism in autoimmune disease is derived from circulating cells and not from cell-free DNA. Fetomaternal microchimerism has been most strongly implicated in the pathogenesis of autoimmune diseases, which have a predilection for women after pregnancy (36) and clinically resemble graft versus host disease (28, 31, 37). Candidate autoimmune diseases with higher prevalence in women include systemic sclerosis (35, 38-40), Sjogren's syndrome (41), thyroid disorders (42), lupus nephritis (34), and systemic lupus erythematosus (43). Microchimerism has now been investigated in many of these diseases, with fetal cells demonstrated in skin lesions, tissues, and peripheral blood, and some results supporting a potential role in disease pathogenesis. Some authors suggest that an explanation for the conflicting results in studies relating fetal microchimerism and autoimmune disease is the migration of fetal cells preferentially into target organs of the disease rather than the circulation (41).

Both microchimeric cells and human leukocyte antigen relationships of host and non-host cells are most likely involved in the subsequent pathogenesis of autoimmune disease (37, 39). The finding of more male cells in women with older sons, as well as the increased amount of microchimerism remote from systemic lupus erythematosus diagnosis and pregnancy, lends support

to the idea that it takes time for the fetal cells to repopulate and establish a cell line (43, 44).

During pregnancy, microchimeric fetal cells have been proven to invade maternal skin, and their presence has been associated with the development of otherwise unexplained inflammatory skin disorders (45). Melanomas that occur during pregnancy have also been shown to include microchimeric fetal cells that cluster around the tumor and appear to have mainly adopted an endothelial phenotype (46). Identification of male–presumed fetal cells in healed maternal cesarean scars after pregnancy suggests that, possibly in response to signals produced by maternal skin injury at surgery, fetal cells migrate to the site of damage to become involved in maternal tissue repair, or proliferate locally (47).

Research has also shown that fetal cells are present in maternal organs affected by non-autoimmune conditions, such as hepatitis C (30), cervical cancer (48), and breast cancer (49). Most investigations that have examined solid tumors convincingly show that fetal cells are preferentially present at tumor sites (28, 50). The function of these cells is unknown, but hypotheses proposed include promotion of tumorigenesis, immunosurveillance, and participation in tissue repair. It cannot be concluded that fetal cell microchimerism always results in a graft-versus-host phenomenon (27).

Furthermore, fetal microchimerism is not always found in association with maternal disease. Because fetomaternal cell trafficking occurs in all pregnancies, microchimerism is likely established in healthy women as well (19, 31, 51). If fetal progenitor/stem cell trafficking occurs in every pregnancy, a much greater incidence of autoimmune disease in postreproductive women would be expected. Therefore, differentiation of fetal stem/progenitor cells engrafted in maternal tissues must occur in response to pre-existing tissue injury such as autoimmunity, or the cells must differentiate into others able to trigger autoimmunity in response to activation events such as infectious disease, malignancy, exposure to chemicals, or tissue injury (19, 27).

Searching for microchimerism in tissues of pregnant women who died due to a variety of causes revealed that Y-chromosome-positive chimeric cells were present in practically all organs, and significantly more so than in organs of nonpregnant women, with the lungs being most chimeric (52). Chimeric cells appeared to be both parenchymal and hematopoietic cells (52). Most interestingly, the distribution of chimeric cells in various organs appears to be similar to those in a mouse model (53), with microchimerism most often present in the lungs (54). This raises the hypothesis of the lungs as a favorable microenvironment for chimerism, a site of passive entrapment, or a site where microchimerism occurs mostly because of the great percentage of cardiac output received in the pulmonary capillary bed. Interestingly, another autopsy study showed that even more chimeric cells in lungs were present in women with preeclampsia (55), a finding that dates back to Schmorl (1, 2) but with the additional evidence of syncytial aggregates containing the anti-angiogenic factor sFlt-1 that may contribute to the systemic endothelial dysfunction characteristic of preeclampsia.

Pregnancy-associated progenitor cells and significance for repair of maternal tissue and organs. From the time that fetal microchimeric cells were identified in pregnant and postpartum women, investigators have explored a range of hypotheses regarding their role with a strong predilection around allo-immunity of these fetal cells (24, 39). An intriguing observation consisted of fetal cells persisting for decades in the maternal body and forming entire thyroid follicles (42). Similarly, when looking more systematically, fetal cells could be shown to adopt phenotypes across multiple lineages in a range of maternal injured tissues (56, 57). This introduced a new paradigm suggesting that fetal cells were stem or progenitors that would engraft in maternal tissues and participate in the naturally occurring injury repair process (58). These fetal stem cells were called pregnancy-associated progenitor cells (PAPCs) (56, 59). Many studies have attempted to understand the functional plasticity and potency of these cells. The initial study in the peripheral blood of pregnant women suggested that PAPCs had hematopoietic as well as lymphoid progenitor activities (CD34+CD38+ phenotype) (24). This was subsequently functionally validated in murine T and B cell immunodeficient models in which fetal lymphoid progenitors could mature in the maternal thymus and bone marrow to form T cells and immunoglobulin-producing B cells (60). Although PAPCs did not demonstrate a significant myeloid potential (61), fetal granulocytes have been reported in maternal blood, suggesting that PAPCs also have myeloid capacity (62).

The multilineage potential of PAPCs supported the role and activity of an early progenitor cell acquired by the mother during the first trimester and early stages of pregnancy. Accordingly, fetal mesenchymal stem cells have been described to enter the circulation and engraft in the maternal bone marrow where they can persist for decades (22, 63). These fetal MSCs have extended plasticity towards mesenchymal lineages. In particular, contribution of PAPCs to mesenchymal lineages has been demonstrated when fibrosis occurs in the kidney, wounds, or the appendix (64–66). Other studies have reported cardiomyocyte differentiation of fetal cells in maternal heart (67), however, muscle differentiation was not reproducible in mice with a maternal genetic defect or injury such as in models of Duchenne muscle dystrophy (68).

Studies attempting to identify PAPCs in placental sections visualized fetal CD34+ in maternal decidual tissues. Surprisingly, most of these cells harbored endothelial markers such as CD31 or von Willebrand factor, suggesting a major contribution of fetal endothelial progenitors to PAPCs (23). Indeed, the endothelial potential of PAPCs has been shown in maternal inflammatory skin diseases (69), skin wounds (47, 70), myocardial infarction (71), melanoma (46), and more recently in stroke (72). In all of these examples, entire blood vessels have formed from fetal microchimeric cells and were connected to maternal vessels. The origin of these endothelial progenitors remains unclear, as some findings suggest that they can emanate from term placentas, and others support the role of earlier CDX2 positive progenitors (71). Evidence for chimeric cells in wound healing comes from a recent study in mice in which it was shown that fetal microchimeric cells with a CD11b+ CD34+ CD31+ phenotype and high expression of the C-C chemokine receptor 2 migrate to areas of maternal injury, where they participate in repair (73). These myeloid cells could be part of the myeloid angiogenic cells that promote wound vascularization via paracrine activities (74). It is suggested that these findings could lead to therapeutic strategies involving tissue repair through natural stem cell therapy.

Finally, beyond these classical mesodermal lineages, several studies have demonstrated the potential of PAPCs to have endodermal or ectodermal fates. Indeed, liver cells have been reported to originate from fetal cell microchimerism (75). Cells derived from multiple nervous system lineages including neurons have been found in postpartum maternal brains or spinal cord (76, 77). Intestinal or thyroid epithelium has been also reported (78). Although the functional significance of these findings is difficult to evaluate, these observations raise fundamental questions in the field of stem cell therapy. Indeed, a major question is whether PAPCs are a homogenous population acquired early during gestation that naturally has multilineage potential or if it is a mixed population including multiple cell types with diverse plasticity.

Maternofetal Microchimerism

MATERNAL CELLS IN OFFSPRING TISSUES

Transfer of cells between the pregnant woman and her fetus also occurs, resulting in persistence of maternal cells in offspring for many years after birth (79–81). Maternal cells have been identified in a variety of tissues including the heart, liver, lung, and brain of neonates (Fig. 2) (82), older infants, and adults, with a variety of potential immunological implications (83, 84). For example, increased levels of maternal microchimerism have been described in many human autoimmune disorders including juvenile dermatomyositis (85), idiopathic inflammatory myopathies (86), and biliary atresia (87–89).

Whether these genetically foreign maternal cells are harmful and instigate alloreactivity, or alternatively provide regenerative properties remains uncertain. Evidence suggesting the latter hypothesis includes the identification of female insulin-producing cells in the pancreatic islets of men with type 1 diabetes (90, 91), keratinocyte phenotypes amongst maternal microchimeric cells in patients with pityriasis lichenoides (92), and cardiac myocyte properties by maternal cells in infants with arrhythmias due to neonatal lupus 3). The rarity of maternal microchimeric cells ($\sim$1 in 10^5 to 10^7 offspring cells) has precluded a more comprehensive systemic identification of their distribution and cellular phenotypes across multiple tissues. Likewise, the lack of tools for their experimental manipulation has prevented definitive identification of their functional roles.

A naturally occurring instance of maternal microchimerism that bypasses these limitations is the enriched accumulation of maternal immune cells in immune-deficient offspring. Graft-versus-host disease from transplacentally engrafted maternal lymphocytes is a well-described clinical manifestation of severe combined immune deficiency (93). On the other hand, expanded levels of circulating maternal T cells can also protect immune-deficient offspring by augmenting antimicrobial host defense against pathogens that cause opportunistic infections (94). In rodents, maternal microchimeric cells have been shown to produce IgG in B cell-deficient offspring (95), and interleukin-2 in the thymus and spleen of interleukin-2-deficient offspring (96).

The absence of defined fetal-neonatal immune cell subsets in these instances undoubtedly creates the physiological niche that allows for microchimerism of transplacentally engrafted maternal leukocytes. Whether microchimeric maternal cells provide similarly protective benefits in the immune components of offspring remains undefined. However, given the relatively naive and hyporesponsive state of fetal and neonatal adaptive immune components that promote increased vulnerability in these developmental windows (97, 98), and the increasingly established purposeful transfer of protective maternal antibodies (99, 100), it would not be surprising that vertically transferred intact maternal cells that persist in offspring also promote the maturation of neonatal immunity.

SIGNIFICANCE OF MATERNAL MICROCHIMERISM FOR THE EDUCATION OF FETAL IMMUNE SYSTEM

Exposure to maternal tissues and the presence of microchimeric maternal cells in the fetus throughout development have important implications for preventing fetal

immune cells from rejecting noninherited maternal alloantigens. For example, it has been shown that in a healthy pregnancy, human fetuses develop regulatory T cells (Tregs) that suppress a fetal T cell response to non-inherited maternal antigens (101). The presence of microchimeric maternal cells may serve to educate fetal T cells toward tolerance, thus avoiding a fetal immune response against the mother. Conversely, this tolerance of the fetus to maternal antigens may be damaged in pregnancy complications such as preterm labor. The cord blood of infants born preterm (due to preterm premature rupture of membranes, a condition in which there is often a subclinical or clinical infection) (102), contains higher levels of maternal cell microchimerism, along with increased maturation of fetal dendritic and T cells (103). Interestingly, cord blood T cells from these neonates are sensitized against maternal antigens and produce tumor necrosis factor-α and interferon-γ, mediators that can contribute to the cascade of uterine contractions in preterm labor (104). These data are consistent with results obtained in mouse models in which there is increased maternal microchimerism after fetal stem cell transplantation (105) or infection with lipopolysaccharide to induce preterm labor (106) or miscarriage (107), and in fetuses undergoing surgery in the womb (108), which is often complicated by preterm labor. Thus, levels of maternal microchimerism may be "tunable" to enable fetal tolerance to maternal antigens at baseline, or sensitization against them during infection/inflammation, to contribute to the complex cascade that can prematurely end the pregnancy.

LONG-TERM IMMUNE CONSEQUENCES OF PREGNANCY/PARTURITION FOR FUTURE PREGNANCIES

Despite the similarities between maternal and fetal cell microchimerism, a critical distinction between them is how these genetically foreign cells establish persistence. This potentially relates to immunological maturity of each respective host. Fetal microchimeric cells enter pregnant women who have a functional repertoire of adaptive immune components. By contrast, maternal cells are found in fetal tissues beginning in the second trimester (108), prior to or during key milestones in human immunological development, such as development of the thymus and acquisition of effector function in peripheral T cells (109, 110).

Exposure to genetically foreign maternal cells in these early stages of immunological development parallels expanded immunological tolerance of offspring to non-inherited maternal antigens (NIMA) (79, 111). Immunological implications of NIMA-specific tolerance have classically been described in transplantation. Before the availability of recombinant erythropoietin, transfusion-dependent individuals broadly exposed to a wide repertoire of genetically foreign human leukocyte antigen alloantigens were found to selectively lack antibodies with NIMA specificity (112). Long-term survival of renal allografts is markedly improved between NIMA matched sibling donor–recipient pairings (113). The risk of graft versus host disease after hematopoietic stem cell transplantation is similarly reduced in NIMA-matched donor–recipient pairings (114–116).

The bidirectional transfer during pregnancy, and persistence of microchimeric cells in mothers and offspring, is highly conserved across mammalian species. Considering the fetus as an allograft, and improved pregnancy outcomes with enforced fetal–allograft tolerance, further highlight potential teleological benefits driving expanded tolerance of offspring to NIMA. An example of these reproductive benefits is reduced susceptibility to fetal wastage in mice during pregnancies in which fetal alloantigens are matched with NIMAs (117). In other words, tolerance to NIMA likely has beneficial impacts in enforcing fetal tolerance during next-generation pregnancies that contain shared fetal alloantigens. Maternal microchimerism is required for these cross-generational reproductive benefits of NIMA-specific tolerance since selective depletion of microchimeric maternal cells in female mice prior to mating eliminates resiliency against fetal wastage in NIMA-fetal alloantigen matched pregnancies (117). This expanded tolerance in mice to non-inherited maternal MHC haplotype antigens parallels classical human observations of reduced sensitization to erythrocyte rhesus (Rh) antigen during pregnancy amongst Rh-negative women born to Rh-positive compared with Rh-negative mothers (118). In particular, pregnancy-induced Rh sensitization was shown to be significantly reduced amongst Rh-negative women with Rh-positive mothers (Rh is transformed into an NIMA) compared with Rh-negative women born to Rh-negative mothers (119). Thus, maternal microchimerism confers important immunological consequences in female offspring on the outcomes of next-generation, future pregnancies.

The similarities between maternal microchimerism in children and fetal microchimerism in mothers raises the question as to whether fetal microchimeric cells retained in women also reinforce fetal tolerance during subsequent pregnancies. Human epidemiology studies consistently show pregnancy complications such as preeclampsia are much more common during first pregnancies (120). For example, an analysis of over 700 000 primiparous mothers showed that the overall 4.1% rate of preeclampsia in a first pregnancy was reduced to 1.7% in subsequent pregnancies (121). Importantly, these protective benefits are partner-specific since a change in paternity overrides the reduced risk of preeclampsia conferred by a prior pregnancy (122–124). Although the necessity of fetal microchimeric cells in sustaining and enforcing fetal tolerance has not been

demonstrated, immunological memory of prior pregnancies, similar to expanded tolerance of offspring to NIMA are each linked with persistent accumulation of immune suppressive regulatory CD4+ T cells with defined antigen-specificity (117, 125).

These reproductive benefits illustrate one aspect of nature's intent in promoting the transfer and retention of microchimeric cells in mothers and their offspring, and also raise interesting questions with regard to potential immunological consequences of individuals being constitutively chimeric. A provocative hypothesis is that defective tolerance to microchimeric cells may drive the underlying pathogenesis of some autoimmune and/or autoinflammatory disorders (84). Important next steps will be to establish the origin, tissue distribution, and molecular properties of microchimeric cells that control whether tolerogenic or sensitization responses are primed to further investigate the role of microhimeric cells in the immune-pathogenesis of idiopathic autoimmune and/or autoinflammatory disorders (79).

SIGNIFICANCE OF MICROCHIMERISM FOR TRANSPLANTATION AND CURE OF GENETIC DISORDERS

Fetal tolerance to non-inherited maternal antigens is transient. Sustaining this tolerance after birth likely depends on the presence of sufficient exposure to maternal cells to enable continued education of T cells. There is one experiment of nature, biliary atresia, in which higher levels of maternal cells are found in the livers of affected children after birth (87–89). Interestingly, there is improved survival of liver transplants from a maternal donor compared to a paternal donor in children with biliary atresia (126), suggesting that the continued presence of maternal microchimerism in this disease can sustain tolerance to the maternal donor.

One of the most exciting applications of fetal tolerance to maternal antigens is in the context of *in utero* stem cell transplantation. Infants with congenital hematopoietic disorders can be cured by hematopoietic stem cell (HSC) transplantation, but a suitable donor is often not available and there are severe complications of the conditioning and immune suppression regimens needed to prepare the recipient's bone marrow for the transplant. However, these disorders are often diagnosed prenatally and could potentially benefit from *in utero* transplantation, a strategy that takes advantage of the unique immune milieu of the fetus to induce tolerance to the transplanted cells. Based on the observation that human fetuses are tolerant to NIMAs during pregnancy (101), and the understanding that the maternal immune system can mediate rejection of third-party cells transplanted into the fetus (104), there is now an ongoing phase 1 clinical trial to transplant maternal HSCs into fetuses with alpha thalassemia major (clinicaltrials.gov NCT02986698). These infants suffer from anemia before birth, requiring antenatal blood transfusions to survive. The HSC transplantation can be given at the same time as the transfusions. If this treatment is found to be safe and effective, it could be expanded to treat patients with similar, more common conditions such as sickle cell anemia. Thus, a better understanding of the biology that enables the trafficking of cells between the mother and the fetus could ultimately result in therapies to treat fetuses with genetic disorders.

SUMMARY AND FUTURE RESEARCH

Intentionally, the focus of this review was on the biological consequences of the exchange of intact cells between the pregnant woman and her fetus during pregnancy. Space limitations did not allow detailed mention of the maternal cells that pass through breast milk after birth (127), or exchange of cell-free nucleic acids, either through free floating fragments of DNA or RNA in the blood, or via structures such as exosomes. It is worth remembering, however, that the twentieth century interest in fetal cell microchimerism originated as a quest to develop noninvasive access to the fetus to facilitate prenatal diagnosis of chromosomal and single gene disorders (4, 5, 128, 129). Although the feasibility of using next generation sequencing in trophoblast cells isolated from maternal blood has been demonstrated using current technologies (130), this is still far from a trial-ready cell-based version of noninvasive prenatal diagnosis. Novel approaches suggested for manipulating first trimester fetal primitive erythroblasts may provide additional exploratory strategies for use of this cell type for noninvasive prenatal diagnosis (131) but improvements in enrichment and automation are necessary for any proposed clinical application (132). Reports using microfluidics to isolate fetal trophoblast cells and erythroblasts show promise (133), as do recent attempts to develop and utilize a single cell-based polymerase chain reaction system to analyze genomic DNA in fetal cells purified from maternal blood (134). In reality, however, it is the use of circulating cell-free fetal (cff) DNA for prenatal screening for aneuploidy that has progressed from an idea to widespread global clinical applications (135) in a very short time, mainly because of the significant quantity of fetal presumed-trophoblast DNA within maternal plasma (136) compared with the rarity of intact fetal cells within maternal blood, as well as their persistence after pregnancy (25). Prenatal screening technologies using cffDNA have limitations (137). Positive predictive values for aneuploidies other than trisomy 21 can be suboptimal based on the testing platform employed and are even lower for common microdeletions. In addition, reliable genome-wide fetal sequencing using cffDNA remains somewhat challenging due to the technological difficulties in detecting small chromosomal imbalances, issues of confined

placental mosaicism, the presence of unexpected maternal chromosomal abnormalities, as well as the high proportion of maternal cell free DNA in plasma (137).

As for the biological consequences of fetal cells in maternal tissues and organs, future investigations in humans need to include complete pregnancy histories, including elective terminations and miscarriages. This is particularly true in the stem cell field, in which the presence of male donor cells in female recipients have been interpreted as transplant successes without mention or consideration of prior pregnancy histories (27). Animal models will continue to be very useful in establishing the mechanisms underlying fetal cell wound healing and/or repair associated with injury and inflammation in the mother. An additional important area for future investigation is whether ongoing stimulation by fetal microchimeric cells in a woman is required for protection against complications primed by prior pregnancy, such as preeclampsia.

The implications of maternal cell microchimerism for the development and education of the fetal immune system, as well as the significance of NIMAs for transplant acceptance, are becoming increasingly appreciated. The role of maternal cells in breast milk may play a role in maintaining NIMA-specific tolerance during childhood and adulthood. This is an area for further research. Microchimerism as a factor in multi-generational reproductive success is another area of interest for the future. Lastly, taking advantage of the priming of the fetal immune system by naturally occurring maternal cell microchimerism to provide lifesaving HSC transplants is an exciting and important clinical application.

In summary, the lifelong consequences of the exchange of cells between a mother and child are profound and have many applications in development, health, and disease. Whereas a mother and her child are undeniably forever connected in many ways, the studies presented here demonstrate that the connection exists at the most basic, granular, cellular level.

Nonstandard Abbreviations: MSC, mesenchymal stem cell; PAPC, pregnancy-associated progenitor cell; NIMA, non-inherited maternal antigen; HSC, hematopoietic stem cell; cff, cell-free fetal.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

K. Khosrotehrani, administrative support, provision of study material or patients; I. Bajema, provision of study material or patients; K. O'Donoghue, administrative support, provision of study material or patients.

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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