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Chimeric Maternal Cells with Tissue-Specific Antigen Expression and Morphology are Common in Infant Tissues

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Abstract

Maternal microchimerism (MMc) has been purported to play a role in the pathogenesis of autoimmunity, but how a small number of foreign cells could contribute to chronic, systemic inflammation has not been explained. Reports of peripheral blood cells differentiating into tissue-specific cell types may shed light on the problem in that chimeric maternal cells could act as target cells within tissues. We investigated MMc in tissues from seven male infants. Female cells, presumed maternal, were characterized by simultaneous immunohistochemistry and fluorescence *in situ* hybridization for X- and Y-chromosomes. Maternal cells constituted 0.017 to 1.9% of parenchymal cells and were found in all infants in liver, pancreas, lung, kidney, bladder, skin, and spleen. Maternal cells were differentiated: maternal hepatocytes in liver, renal tubular cells in kidney, and β -islet cells in pancreas. Maternal cells were not found in areas of tissue injury or inflammatory infiltrate. Maternal hematopoietic cells were found only in hearts from patients with neonatal lupus. Thus, differentiated maternal cells are present in multiple tissue types and occur independently of inflammation or tissue injury. Loss of tolerance to maternal parenchymal cells could lead to organ-specific "auto" inflammatory disease and elimination of maternal cells in areas of inflammation.

Keywords

fetal development; maternal microchimerism; differentiation; stem cells; pregnancy

Introduction

Bidirectional cell transfer between mother and fetus was first recognized decades ago, and is now known to be a common event (1,2). The persistence of transferred cells has been shown to lead to long-term maternal and fetal microchimerism in the blood of the child and the mother, respectively (3–6). Subsequent studies demonstrated that both fetal and maternal microchimerism can also be found in tissues from patients with autoimmune diseases or

congenital anomalies (7–13). The frequency and phenotypes of MMc in normal tissues, however, has not been reported.

Iatrogenic chimerism occurs in transplantation, and when graft-versus-host disease (GVHD) was first recognized as a complication of hematopoietic cell transplantation, its similarities to autoimmune diseases were described (14,15). Naturally acquired microchimerism has thus been hypothesized to be involved in the pathogenesis of autoimmune diseases (16). MMc in the newborn is likely benign or may be beneficial, but may also transmit malignancy or cause GVHD (17). Fetal microchimerism (FMc) has been investigated in a number of autoimmune diseases including systemic sclerosis, primary biliary cirrhosis, thyroid disease, and systemic lupus erythematosus (5).

Long term MMc was first reported in immune competent individuals and in the peripheral blood of SSc patients and healthy subjects (4) and has since been identified in additional SSc patients (18) and in the target organs and blood in neonatal lupus syndrome (NLS) (12) and myositis (7,8). In an early study, maternal DNA was found to be *increased* in prevalence in and levels in blood in patients with systemic sclerosis (18). MMc was found in tissues that were targets of disease in one patient, but also in tissues that were not involved. That the levels of MMc were 10 to 20-fold higher in the tissues than in the blood suggests that future studies into the mechanisms for the role of maternal cells in inflammatory disease may be best directed toward the parenchymal and immunological cells within the target organs. In older children, two groups have reported increased female cells (presumed maternal) in muscle biopsies from male patients with idiopathic myositis compared to controls (7,8). Moreover, MMc in the blood was also increased in myositis patients, as detected by nested PCR assay for maternal HLA alleles not shared with the patient (7). MMc has also been demonstrated in pityriasis lichenoides, where female cells in the form of keratinocytes were found in 11 of 12 male patients in skin biopsies (19). T lymphocytes, antigen presenting cells and B lymphocytes have been found in the affected skin of localized scleroderma patients, but also controls (20). Thus, it is not clear what variety of allogeneic cell roles maternal cells may play in the blood or tissues of children with autoimmune diseases. MMc is not found in every suspected disease – for instance, infantile hemangioma, hypothesized to be placental-derived maternal endothelial cells, was investigated but not found associated with MMc (21).

In GVHD interactions between host and non-host cells lead to chronic inflammation. Because there is usually genetic disparity between maternal and fetal cells, naturally acquired microchimerism could also potentially lead to chronic inflammation. However, although levels of MMc and FMc have been shown in some studies to be higher in patients with autoimmune diseases, both FMc and MMc are common in the blood of healthy controls. Also, unlike GVHD, in which the dominant leukocyte population is foreign, blood maternal and fetal microchimerism exists at very low levels (one chimeric cell per 10^4 to 10^6 host cells). Therefore, it is difficult to envision a mechanism by which such a tiny number of foreign cells would contribute to autoimmune disease as immune effector cells. An alternative possibility is that tissue-specific microchimeric cells act as target cells, stimulating chronic inflammation, as in a low-level chronic infection.

The phenotypes of microchimeric maternal cells in tissues have not been well-characterized. Because only circulating cells pass between the mother and fetus, the most predictable phenotype would be hematopoietic. Transplanted circulating hematopoietic stem cells, however, can differentiate into tissue-specific cells and take up residence in the new host organs as hepatocytes, neurons, myocytes, and endothelial cells forming blood vessels (22). In previous studies we identified maternal cells with the phenotypes of myocardial cells in infants with neonatal lupus syndrome (NLS) who died of congenital heart block and into β -islet cells in pancreas with and without type I diabetes, suggesting the possibility of maternal cells

differentiating in situ within fetal organs (12,13). In the present study we investigated multiple other tissue types for MMc and asked whether maternal cells were infiltrating inflammatory cells or were differentiated cells incorporated within the tissue architecture. We also examined the milieu of chimeric maternal cells to ask if MMc is associated with inflammation.

Methods

Tissue Samples

The Seattle Children's Hospital and Fred Hutchinson Cancer Research Center Internal Review Boards approved the protocol. All samples studied derived from seven autopsied male subjects obtained from the archives of the Department of Pathology at the Children's Hospital that were used previously for a related study (12). Only male subjects were used, because the approach employed identifies sex chromosome markers distinguishing female (presumed maternal) cells in male (child) tissue. Table 1 includes clinical and pathologic data for each subject. The ages of the subjects ranged from birth to 4.5 months. Five of the seven subjects were born prematurely (27 to 36 weeks gestation). Three subjects had died of NLS congenital heart block (subjects 1–3), one who also had thrombocytopenia and hepatitis (subject 2), another Parvovirus (subject 3). The other subjects died of sepsis, fetal hydrops, and congenital anomalies (subjects 4–7). Subjects 2, 5, and 6 had transfusions of irradiated blood. Because of the archival nature of the specimens, not all clinical history or tissue types were available for all subjects.

Simultaneous fluorescence in situ hybridization (FISH) and immunohistochemistry to phenotype maternal cells

Tissue sections from all available organs for each subject were first subjected to two-color immunohistochemistry with two different specific antibodies; the same section was then probed for X- and Y-chromosomes. The antibody stains were retained throughout the FISH procedure, so that light microscopy and fluorescence microscopy could be used simultaneously to characterize female cells in male tissues. The method has an advantage over previous methods, in which adjacent tissue sections were probed for X- and Y-chromosomes and tissue-specific antigens, and estimates were made to determine the phenotypes of specific cells based on location and surrounding morphology. Table two lists the antigens targeted to specific tissue types. Antibodies to CD45 were used to identify hematopoietic cells (DAKO, Carpinteria, CA), insulin for β -islet cells (ICN Biomedicals, Inc, Irvine, CA), glucagon for α -islet cells (Sigma-Aldrich, St. Louis, MO), CAM 5.2 or AE3 for cytokeratins on hepatocytes, cholangiocytes, and renal tubular cells (BD-Pharmingen, San Diego, CA) and sarcomeric α -actin for myocytes (Sigma-Aldrich). Immunohistochemistry was performed with a streptavidin-conjugated secondary antibody-mediated peroxidase development system (Elite Universal Vectastain ABC kit, Vector Laboratories, Burlingame, CA), and developed with substrates SG (Vector Laboratories, blue-gray in color), and DAB (BioFX Labs, Owing Mills, MD). A significant strength of this approach is that it includes an additional internal control, since cells staining for tissue-specific antigens can readily be shown to *not* express CD45, which was used for each tissue in combination with antibodies for parenchymal cells, and therefore not to be overlying hematopoietic cells, and vice versa.

The FISH method previously described was used to probe the tissue sections for X- and Y-chromosome sequences (12). The probe for the X-chromosome centromere-associated marker DXZ1 was labeled with orange fluorescent Cy3-linked dUTP (Amersham-Pharmacia, Piscataway, NJ). The probe for the Y-chromosome satellite sequence DYZ1 was labeled with green fluorescent FITC-linked dUTP (Roche Laboratories, Indianapolis, IN). The sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA) with DAPI (4',6'-diamidino-2-phenylindole hydrochloride, Sigma-Aldrich, 100 ng/ml), then analyzed by

fluorescence microscopy using a triple band filter specific for fluorescein, Cy3 and DAPI. The tissues were visualized with a NIKON LABOPHOT-2 microscope and photographs taken with a NIKON Coolpix 995 camera. The photos were processed using Adobe Photoshop Elements on a Macintosh G3. Photographs were edited using only cropping and the brightness/contrast and white point/black point functions for the full photographs (not selected areas). In some cases photographs taken with the green filter were overlaid with the red-green-blue filter.

To quantify the microchimeric cells, at least 2,000 nuclei from each tissue section were scored. Only clearly-defined nuclei with two obviously distinctive signals (XX or XY) were counted. Nuclei with more than two signals (e.g., XXX, XXXX) were rarely observed in pancreas and liver, but were not believed to be products of fusion, as we never observed XXXY cells. Moreover, tetraploid hepatocytes and pancreatic cells have been reported, but were usually binucleate, twice as large as monoploid cells, and found at older ages (23,24). The efficiency of the FISH probes, as defined by the percentage of all nuclei with two visible sex chromosome signals, varied with specific sections and tissues, because of different fixation times prior to paraffinization, IHC conditions, and tissue fragility. The efficiency of FISH signals for each tissue and target antigen is shown in Table 2.

Results

Female cells, presumed maternal, were identified in all tissues examined and in all subjects, including the liver, kidney, pancreas, lung, bladder, skin, and spleen. We have previously shown MMc in the heart (12). The percentages of maternal cells within each cell type are summarized in Figure 1. In the liver, six of seven subjects tested had female nuclei with two clearly-distinguished X-chromosomes, and female nuclei constituted 0.05 to 0.2% of all nuclei examined. The kidney harbored maternal cells at a level of 0.1 to 0.9%. In the pancreas two of four subjects had maternal cells at levels of 0.05 to 0.25%. Maternal cells were found in the lung at levels of 0.05 to 0.1% and in the skin and bladder at 0.05%. Because the liver and the spleen are hematopoietic organs in the fetus, we considered whether maternal hematopoietic cells might home to these organs. Only one of four spleens, however, harbored maternal cells.

To determine the phenotypes of the maternal cells within infant tissues, we used FISH for X- and Y-chromosomes with immunohistochemistry for specific cell types. In the liver we stained for cytokeratins expressed by hepatocytes and biliary epithelia. We found that 42% of the maternal cells identified expressed the cytokeratin recognized by this antibody (Figure 2A–B). The maternal cells had the morphology of hepatocytes, and were not in the vicinities of biliary ducts. In these tissue sections, as in other tissues, an anti-CD45 antibody served as an internal control for hematopoietic cells, confirming that the maternal hepatocytes did not express CD45, and thus were not hematopoietic cells overlying the hepatocytes. Maternal hepatocytes were identified in six of seven subjects, and ranged from one in 6000 to three in 2,000 hepatocytes assayed (0.017–0.15%).

Studies of the lung employed immunohistochemistry with antibodies to epithelial cells, endothelial cells, and type II pneumocytes. One maternal cell expressed surfactant protein B, and thus was a type II pneumocyte (not shown) and another maternal cell expressed cytokeratin, suggesting it is an epithelial cell (Figure 2C–D). Other maternal cells in the lung did not stain for surfactant, the endothelial cell marker Von Willebrand Factor or for the epithelial cell marker cytokeratin recognized by the AE3 antibody, and thus remain uncharacterized. In the heart most of the maternal cells expressed sarcomeric α -actin, as previously reported (Figure 2E–F) (25), but some expressed neither CD45 nor sarcomeric α -actin, and thus could be endothelial cells, pericytes, fibroblasts, epicardium, valve tissue or stem cells (Figure 2G–H).

In the kidney, two different antibodies specific for cytokeratins expressed by renal tubular epithelial cells were used (CAM5.2 and AE3). All maternal cells identified were incorporated into the structure of renal tubular cells (found in two of three subjects for which kidney tissue was available, Figure 3A–C). Chimeric maternal cells all expressed cytokeratins, and had the morphology typical of tubular epithelial cells. Maternal tubular epithelial cells were present at levels of 0.025–0.3% of host epithelial cells. In the pancreas maternal β -islet cells were identified by immunohistochemistry for insulin expression within the cytoplasm and α -cells by glucagon expression (Figure 3D, E). Islet β cells expressing insulin were clearly distinguished from the α -islet cells expressing glucagon by virtue of different substrate colors within the same fields. Among islet β cells up to 1.9% were maternal. No maternal cells were identified that expressed glucagon.

Maternal hematopoietic cells, expressing either CD45 or CD3, were found only rarely in the hearts affected by neonatal lupus syndrome, and not in any other tissues. In order to determine if MMc is associated with inflammation, as would be expected if maternal cells were targets of host immunity, we examined adjacent sections stained with hematoxylin and eosin. Active inflammation was defined by areas where neutrophils or mononuclear cells were infiltrating areas where they do not usually reside. This was detected in the presence or absence of tissue destruction or fibrosis. Areas with lymphoid tissue or extramedullary hematopoiesis, were not considered to be inflammatory. In the liver, chronic inflammatory infiltrates were present in two subjects, and extramedullary hematopoiesis in six subjects (Table 1). MMc was not found in any of these areas with hematopoietic cells, but rather in all organs was found in areas of normal, unperturbed tissue.

Discussion

We have demonstrated that maternal cells that presumably passed into the fetus during pregnancy can home to tissues and resemble myocardial, hepatic, renal, pulmonary, and pancreatic cells by morphology and tissue-specific expression. The highest levels were in liver, kidney, and pancreas. Previous studies have demonstrated maternal cells in the blood and tissues, but early attempts to characterize them described hematopoietic cells (7,8,26). In contrast, we found mostly parenchymal and only rare maternal hematopoietic cells in tissues.

How maternal parenchymal cells come to be incorporated into a child's tissues is not known. The maternal microchimerism was of mesoderm, endoderm and ectodermal cell types. Thus, maternal stem cells could have engrafted during the first trimester and differentiated alongside the child's cells. Alternatively, the tissue-specific maternal cells could have been derived from circulating maternal stem cells that transdifferentiated into organ tissues, as has been suggested in the setting of stem cell transplantation (22). Circulating maternal cells could have been precursors to tissue-specific cells or they could have differentiated after migrating into the tissues.

Another possibility that cannot be ruled out in the current study is that maternal cells are not actually differentiated tissue-specific cells, but rather hematopoietic cells that have fused with fetal cells, a phenomenon that has been described *in vitro* (27,28) and *in vivo*, which can result in multinucleated cells in which each nucleus appears to be a normal diploid nucleus (29,30). In the latter mouse model, transplanted hematopoietic stem cells fused with hepatocytes and expressed genes at a high enough level to replace an enzyme deficiency. Maternal cells could likewise fuse with a child's cells leading to expression of maternal HLA molecules, which could be recognized by the child's immune system as being foreign. Thus, although it is not possible in our study to determine whether the maternal cells have differentiated or rather fused with fetal cells, even fused maternal-fetal cells could be pathogenic. In any case, the rate of naturally-occurring MMc appears not to be of the quality and/or quantity to replace functional

host cells in cases of congenital enzyme deficiencies, as has been demonstrated for transplanted cells (30). Whether MMc could be potentiated to restore fetal deficiencies is not known, but would be advantageous because of the inherent immune tolerance to maternal antigens.

The female cells found in male tissues were presumed to be maternal, but there are other potential sources of female cells. Twin/twin transfusion is one alternative source of microchimerism. The subjects in this study had no known twins, but the vanishing twin phenomenon is not unusual (31). Also, female cells from a previous fetus carried by the mother could persist through a subsequent pregnancy, and then pass into the subject fetus. Three subjects had received blood transfusions, which can lead to microchimerism (32), but chimerism from female blood donors is unlikely, because blood transfused into newborns is routinely irradiated to eliminate live cells. Therefore, the female cells detected in newborn tissues in this study are most likely to be of maternal origin. Definitive evidence for the source of female cells could be obtained if maternal DNA were available from the mothers by detection of non-inherited, non-shared maternal alleles of polymorphic genes, as has been employed previously in blood samples (2,4,18,33).

That MMc is common in the absence of inflammatory disease implies a normal lasting tolerance to maternal antigens. Such anti-maternal T lymphocyte tolerance has been demonstrated in a mouse model, with the requirement for nursing (34), perhaps through mechanisms of oral tolerance to maternal HLA proteins in the milk (35). The mouse, however, is a limited model for events occurring during human pregnancy, because of the physiological, immunological and anatomical differences between species (36,37).

Maternal microchimerism could contribute to autoimmune diseases or participate in a secondary event in pathogenesis, amplified as part of an attempt to regenerate damaged tissue, as has been described for circulating stem cells in myocardial injury (38–41), diabetes (42), and ischemic renal injury (43). Stem cell-derived tissue may be beneficial, improving organ function (30,44,45). An understanding of the mechanisms of tissue regeneration by adult stem cells may lead to new cellular treatments for organ failure through delivery of new stem cells to aid in tissue regeneration (30,46,47). That MMc is tolerated by the host immune system suggests the potential for maternal stem cells as donor cells in tissue regeneration protocols. Because autoimmune disease is also characterized by tissue injury and regeneration, a similar mechanism of stem cell recruitment may contribute to organ repair in these patients. Recruited precursor stem cells could be of maternal origin, as MMc has been identified in the murine bone marrow (48–51). However, many of the tissues included in our study were not injured, and thus would only have experienced normal cell turnover rather than overall regeneration, yet contained maternal cells, suggesting that MMc within tissues may be a normal phenomenon. Similar observations have been made for fetal microchimerism, which has been found in multiple tissue types, both healthy and targets of autoimmunity (52,53).

In summary, maternal cells that travel into a fetus can migrate into the tissues that are target organs for autoimmune disease and express tissue-specific proteins. We found maternal microchimerism in tissues was frequent, present in most subjects and representing between 0.05 and 0.9% of all cells and up to 1.9% of specific differentiated cell types. MMc was not necessarily associated with inflammation. Chimeric maternal cells may play different roles during different times of development – early in gestation benign and simply part of normal tissue differentiation; later recognized as foreign, and thus immunostimulatory. Future studies will be necessary to determine how widespread this phenomenon is at older ages and the beneficial or deleterious roles that maternal cells may play in children.

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Abbreviations

MMc	maternal microchimerism
FISH	fluorescence in situ hybridization
IHC	immunohistochemistry
NLS	neonatal lupus syndrome
GVHD	graft versus host disease
VWF	Von Willebrand Factor
G	gestation
ANA	antinuclear antibodies
SSA	antibodies to SSA antigen (Ro)
SSB	antibodies to SSB antigen (La)
RF	rheumatoid factor
SLE	systemic lupus erythematosus
Parvo PCR+	Parvovirus DNA detected by PCR in blood from the mother
CHB	congenital heart block
ASD	atrioseptal defect
VSD	ventricular septal defect
DIC	disseminated intravascular coagulation
EFE	endocardial fibroelastosis
AV	atrioventricular
N/A	not available

References

- Desai RG, Creger WP. Maternofetal Passage of Leukocytes and Platelets in Man. *Blood* 1963;21(6): 665–673. [PubMed: 14027196]
- Lo YM, Lau TK, Chan LY, Leung TN, Chang AM. Quantitative analysis of the bidirectional fetomaternal transfer of nucleated cells and plasma DNA. *Clin Chem* 2000;46(9):1301–1309. [PubMed: 10973858]
- Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A* 1996;93(2):705–708. [PubMed: 8570620]
- Maloney S, Smith A, Furst DE, Myerson D, Rupert K, Evans PC, et al. Microchimerism of maternal origin persists into adult life. *J Clin Invest* 1999;104(1):41–47. [PubMed: 10393697]

5. Adams KM, Nelson JL. Microchimerism: an investigative frontier in autoimmunity and transplantation. *JAMA* 2004;291(9):1127–1131. [PubMed: 14996783]
6. Stevens AM. Maternal microchimerism--allogeneic target of autoimmune disease or normal biology? *Clinical and Applied Immunology Reviews* 2005;5:325–338.
7. Reed A, Picnorell YJ, Harwood A, Kredich D. Chimerism in children with juvenile dermatomyositis. *Lancet* 2000;356:2156–2157. [PubMed: 11191546]
8. Artlett CM, Ramos R, Jiminez SA, Patterson K, Miller FW, Rider LG. Chimeric cells of maternal origin in juvenile idiopathic inflammatory myopathies. Childhood Myositis Heterogeneity Collaborative Group. *Lancet* 2000;356(9248):2155–6. [PubMed: 11191545]
9. Johnson KL, Nelson JL, Furst DE, McSweeney PA, Roberts DJ, Zhen DK, et al. Fetal cell microchimerism in tissue from multiple sites in women with systemic sclerosis. *Arthritis Rheum* 2001;44(8):1848–1854. [PubMed: 11508438]
10. Johnson KL, McAlindon TE, Mulcahy E, Bianchi DW. Microchimerism in a female patient with systemic lupus erythematosus. *Arthritis Rheum* 2001;44(9):2107–2111. [PubMed: 11592373]
11. Srivatsa B, Srivatsa S, Johnson KL, Bianchi DW. Maternal cell microchimerism in newborn tissues. *J Pediatr* 2003;142(1):31–35. [PubMed: 12520251]
12. Stevens AM, Hermes HM, Rutledge JC, Buyon JP, Nelson JL. Myocardial-tissue-specific phenotype of maternal microchimerism in neonatal lupus congenital heart block. *Lancet* 2003;362(9396):1617–1623. [PubMed: 14630442]
13. Nelson JL, Gillespie KM, Lambert NC, Stevens AM, Loubiere LS, Rutledge JC, et al. Maternal microchimerism in peripheral blood in type 1 diabetes and pancreatic islet beta cell microchimerism. *Proc Natl Acad Sci U S A* 2007;104(5):1637–42. [PubMed: 17244711]
14. Fialkow PJ, Gilchrist C, Allison AC. Autoimmunity in chronic graft-versus-host disease. *Clin Exp Immunol* 1973;13(4):479–486. [PubMed: 4123818]
15. Rouquette-Gally AM, Boyeldieu D, Gluckman E, Abuaf N, Combrisson A. Autoimmunity in 28 patients after allogeneic bone marrow transplantation: comparison with Sjogren syndrome and scleroderma. *Br J Haematol* 1987;66(1):45–47. [PubMed: 3297128]
16. Nelson JL. Maternal-fetal immunology and autoimmune disease: is some autoimmune disease auto-alloimmune or allo-autoimmune? *Arthritis Rheum* 1996;39(2):191–194. [PubMed: 8849367]
17. Stevens AM. Do maternal cells trigger or perpetuate autoimmune diseases in children? *Pediatr Rheumatol Online J* 2007;5:9. [PubMed: 17550578]
18. Lambert NC, Erickson TD, Yan Z, Pang JM, Guthrie KA, Furst DE, et al. Quantification of maternal microchimerism by HLA-specific real-time polymerase chain reaction: studies of healthy women and women with scleroderma. *Arthritis Rheum* 2004;50(3):906–914. [PubMed: 15022334]
19. Khosrotehrani K, Guegan S, Fraïtag S, Oster M, de Prost Y, Bodemer C, et al. Presence of chimeric maternally derived keratinocytes in cutaneous inflammatory diseases of children: the example of pityriasis lichenoides. *J Invest Dermatol* 2006;126(2):345–8. [PubMed: 16374466]
20. McNallan KT, Aponte C, el-Azhary R, Mason T, Nelson AM, Paat JJ, et al. Immunophenotyping of chimeric cells in localized scleroderma. *Rheumatology (Oxford)* 2007;46(3):398–402. [PubMed: 17085771]
21. Pittman KM, Losken HW, Kleinman ME, Marcus JR, Blei F, Gurtner GC, et al. No evidence for maternal-fetal microchimerism in infantile hemangioma: a molecular genetic investigation. *J Invest Dermatol* 2006;126(11):2533–8. [PubMed: 16902414]
22. Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. *Blood* 2003;102(10):3483–3493. [PubMed: 12893756]
23. Watanabe T, Tanaka Y. Age-related alterations in the size of human hepatocytes. A study of mononuclear and binucleate cells. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1982;39(1):9–20. [PubMed: 6123185]
24. Ehrie MG, Swartz FJ. Diploid, tetraploid and octaploid beta cells in the islets of Langerhans of the normal human pancreas. *Diabetes* 1974;23(7):583–8. [PubMed: 4135223]
25. Pollack MS, Kirkpatrick D, Kapoor N, Dupont B, O'Reilly RJ. Identification by HLA typing of intrauterine-derived maternal T cells in four patients with severe combined immunodeficiency. *N Engl J Med* 1982;307(11):662–666. [PubMed: 7050708]

26. McNallan KT, Aponte C, El-Azhary R, Mason T, Nelson AM, Paat JJ, et al. Immunophenotyping of chimeric cells in localized scleroderma. *Rheumatology (Oxford)*. 2006
27. Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 2002;416(6880):542–545. [PubMed: 11932747]
28. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. *Nature* 2002;416(6880):545–548. [PubMed: 11932748]
29. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al Dhalimy M, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003;422(6934):897–901. [PubMed: 12665832]
30. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 2003;422(6934):901–904. [PubMed: 12665833]
31. Hall JG. Twinning. *Lancet* 2003;362(9385):735–743. [PubMed: 12957099]
32. Lee TH, Paglieroni T, Ohto H, Holland PV, Busch MP. Survival of donor leukocyte subpopulations in immunocompetent transfusion recipients: frequent long-term microchimerism in severe trauma patients. *Blood* 1999;93(9):3127–3139. [PubMed: 10216112]
33. Loubiere LS, Lambert NC, Flinn LJ, Erickson TD, Yan Z, Guthrie KA, et al. Maternal microchimerism in healthy adults in lymphocytes, monocyte/macrophages and NK cells. *Lab Invest* 2006;86(11):1185–92. [PubMed: 16969370]
34. Andrassy J, Kusaka S, Jankowska-Gan E, Torrealba JR, Haynes LD, Marthaler BR, et al. Tolerance to noninherited maternal MHC antigens in mice. *J Immunol* 2003;171(10):5554–5561. [PubMed: 14607963]
35. Molitor ML, Haynes LD, Jankowska-Gan E, Mulder A, Burlingham WJ. HLA class I noninherited maternal antigens in cord blood and breast milk. *Hum Immunol* 2004;65(3):231–239. [PubMed: 15041161]
36. Malassine A, Frendo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. *Hum Reprod Update* 2003;9(6):531–9. [PubMed: 14714590]
37. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol* 2004;172(5):2731–8. [PubMed: 14978070]
38. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 2001;98(18):10344–10349.
39. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7(4):430–436. [PubMed: 11283669]
40. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, et al. Chimerism of the transplanted heart. *N Engl J Med* 2002;346(1):5–15.
41. LaBarge MA, Blau HM. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* 2002;111(4):589–601. [PubMed: 12437931]
42. Kodama S, Kuhlreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* 2003;302(5648):1223–1227. [PubMed: 14615542]
43. Poulsom R. Does bone marrow contain renal precursor cells? *Nephron Exp. Nephrol* 2003;93(2):e53.
44. Wang Y, Iwatani H, Ito T, Horimoto N, Yamato M, Matsui I, et al. Fetal cells in mother rats contribute to the remodeling of liver and kidney after injury. *Biochem Biophys Res Commun* 2004;325(3):961–7. [PubMed: 15541383]
45. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R. Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. *Proc Natl Acad Sci U S A* 2006;103(19):7321–6. [PubMed: 16648256]
46. Hennessy B, Korbly M, Estrov Z. Circulating stem cells and tissue repair. *Panminerva Med* 2004;46(1):1–11. [PubMed: 15238877]

47. Korbly M, Estrov Z, Champlin R. Adult stem cells and tissue repair. *Bone Marrow Transplant* 2003;32 (Suppl 1):S23–4. [PubMed: 12931235]
48. Tuffrey M, Bishun NP, Barnes RD. Porosity of the mouse placenta to maternal cells. *Nature* 1969;221 (185):1029–1030. [PubMed: 5774393]
49. Piotrowski P, Croy BA. Maternal cells are widely distributed in murine fetuses in utero. *Biol Reprod* 1996;54(5):1103–1110. [PubMed: 8722632]
50. Arvola M, Gustafsson E, Svensson L, Jansson L, Holmdahl R, Heyman B, et al. Immunoglobulin-secreting cells of maternal origin can be detected in B cell-deficient mice. *Biol Reprod* 2000;63(6): 1817–1824. [PubMed: 11090454]
51. Marleau AM, Greenwood JD, Wei Q, Singh B, Croy BA. Chimerism of murine fetal bone marrow by maternal cells occurs in late gestation and persists into adulthood. *Lab Invest* 2003;83(5):673–681. [PubMed: 12746477]
52. Koopmans M, Hovinga IC, Baelde HJ, Fernandes RJ, de Heer E, Bruijn JA, et al. Chimerism in kidneys, livers and hearts of normal women: implications for transplantation studies. *Am J Transplant* 2005;5(6):1495–502. [PubMed: 15888060]
53. Khosrotehrani K, Bianchi DW. Multi-lineage potential of fetal cells in maternal tissue: a legacy in reverse. *J Cell Sci* 2005;118(Pt 8):1559–63. [PubMed: 15811948]

Figure 1. Quantification of maternal cells in organs from seven infants

Maternal cells were identified by FISH for X- and Y-chromosomes. Female (presumed maternal) nuclei with two red X-chromosomes were identified among the infant's male nuclei with one red X-chromosome and one green Y-chromosome. At least 2,000 nuclei with two clearly-defined sex chromosomes were examined per slide. Each bar represents one tissue section on an individual slide. X- axes refer to subject number; Y-axes, MMc as percentage of host cells.

Figure 2. Maternal cells characterized in infant tissues

Immunohistochemistry was performed with antibodies to tissue-specific proteins followed by FISH for X- and Y-chromosomes. Arrows designate female (presumed maternal) nuclei with two red X-chromosomes among the infant's male nuclei, each with one red X-chromosome and one green Y-chromosome.

A, B. One maternal hepatocyte expressed cytokeratin (brown) throughout the cytoplasm surrounding the maternal nucleus incorporated into the hepatic tissue structure (20X, inset 100X).

C, D. Maternal epithelial cell in the lung identified by cytokeratin expression (brown, arrows) but not the endothelial cell marker Von Willebrand Factor (blue-grey stain, white arrowhead). (40X)

E, F. Maternal myocardial cell identified by expression of sarcomeric α -actin (brown). The maternal cell does not express CD45 (blue-grey), which serves as an additional internal control, confirming that the maternal cell is not a hematopoietic cell overlying a myocardial cell. (40X)

G, H. Maternal cell in the myocardium that does not express CD45 or sarcomeric α -actin. (40X)

Figure 3. Maternal cells characterized in infant tissues

Maternal cells were identified as per methods described in Figure 2.

A. A maternal (female) cell in a cross-section of a renal tubule was identified by FISH. (40X)

B, C. Immunohistochemistry was performed with antibodies to cytokeratin (brown) and CD45 (blue-gray). A representative maternal cell stained with antibodies to cytokeratin (brown) and was incorporated into a renal tubule (40X, inset 100X).

C, D. Maternal β -islet cell in the pancreas. Immunohistochemistry was performed with antibodies to insulin (brown), and glucagon (grey). Insulin expression is seen throughout the tissue and in the cytoplasm surrounding the maternal nucleus (arrowhead, 40X).

Clinical characteristics of study subjects

Subject	1	2	3	4	5	6	7
Gestational Age(weeks)	40	31	36	27	32	36	37
Age at Death	2 hours	8 days	18	Birth	8 days	1 day	1 day
Maternal Age (years)	30	29	22	N/A	N/A	25	30
Maternal History	G1 ANA, SSA Healthy	G2 ANA, RF, SSB SLE diagnosed during pregnancy	G3 ANA, SSA, Parvo PCR+	Hypertension	Healthy, normal pregnancy and delivery	G1 Healthy, normal pregnancy and delivery	G1 Fever, Group B Streptococcus
Problems	CHB, ASD, pericardial, pleural effusions	CHB, meconium aspiration, hyperbilirubinemia thrombocytopenia	CHB, peritonitis, sepsis	Fetal hydrops, ASD	Aortic atresia, VSD	Diaphragmatic hernia, cardiopulmonary arrest, amniotic bands, intraventricular hemorrhage	Group B Streptococcus sepsis, neutropenia, cerebral edema, pneumonia, DIC, gastroesophageal ulcers
Treatments		Exchange transfusions, corticosteroids	Corticosteroids, bowel resection	None	Transfusion	Transfusion	Antibiotics
Pathology							
Liver	Portal chronic inflammation with bridging fibrosis, extramedullary hematopoiesis	Cholestasis, moderate extramedullary hematopoiesis	Mild chronic inflammatory cells in portal tract, bridging fibrosis	Extensive extramedullary hematopoiesis, congestion, fibrosis, necrosis	Minor extramedullary hematopoiesis, no inflammation	Mild extramedullary hematopoiesis, extramedullary hematopoiesis	Focal residual extramedullary hematopoiesis
Heart	EFE, fibrosis, calcification; lymphohistiocytic inflammatory infiltrate with rare giant cells around degenerating myocytes	AV node fibrosis, calcification of cardiac system, no myocarditis	AV node fibrosis, calcification, lymphohistiocytic inflammatory giant cells	Disarray, edema, EFE	necrosis	Few mononuclear cells dispersed throughout the tissue	Focal interstitial hemorrhage in ventricles
Kidney	Normal	N/A	Normal	N/A	N/A	Congestion	N/A
Spleen	Normal	N/A	Congestion	Congestion	Normal	Hemosiderosis	Hemosiderosis
Pancreas	Islet cell hyperplasia	N/A	N/A	Normal	Normal	Mild fibrosis, prominent localized lymphoid tissue, not inflammatory	N/A
Lungs	N/A	Meconium aspiration without inflammation, consistent with hypoxia	Macrophagic pneumonitis consistent with congestive heart failure	N/A	Congestion	Pleural petechiae, focal hemorrhages, pneumonia	N/A
Bladder	N/A	Normal	N/A	N/A	N/A	N/A	N/A
Skin	N/A	Normal	N/A	N/A	N/A	N/A	N/A
Placenta	Succentuate lobe, chorangiosis	N/A	N/A	Edema with dystrophic mineralization of villae; nodules, possible squamous metaplasia, intravillous hematomas	N/A	Normal	Group B Streptococcus cultured

G, gestation; ANA, antinuclear antibodies; SSA, antibodies to SSA antigen (Ro); SSB, antibodies to SSB antigen (La); RF, rheumatoid factor; SLE, systemic lupus erythematosus; Parvo PCR+, Parvovirus DNA detected by PCR in blood from the mother; CHB, congenital heart block; ASD, atrioseptal defect; VSD, ventricular septal defect; DIC, disseminated intravascular coagulation; EFE, endocardial fibroelastosis; AV, atrioventricular; N/A, not available.

Table 2

Efficiency of FISH for X- and Y-chromosomes (percent of nuclei with two distinct sex chromosomes) after immunohistochemistry in different tissue types. The efficiency depends on fixation conditions of the tissue, the antibody used, and the tissue type.

Tissue	Antigen	Cell Type	Efficiency of FISH for X and Y*
All	CD45	Hematopoietic	90%
Kidney	Cytokeratins	Tubular epithelial	84%
Kidney	VWF	Endothelial	80%
Lung	SPB	Alveolar epithelial cells	60%
Pancreas	Glucagon	α -islet cells	65%
Pancreas	Insulin	β -islet cells	49%
Liver	Cytokeratins	Hepatocytes	67%

VWF, Von Willebrand Factor; SPB, surfactant protein B.