Isolation of fetal cells from maternal blood is under active investigation as a noninvasive method of prenatal diagnosis. In the context of studying cell surface antigens expressed on fetal cells we discovered that fetal cells from a prior pregnancy also could be detected. This led to the appreciation of the persistence of fetal cells in maternal blood for as long as 27 years postpartum, and the realization that following pregnancy, a woman becomes a chimera. Quantitative polymerase chain reaction analyses have shown that a term pregnancy is not required for the subsequent development of fetal cell microchimerism. As many as 500,000 fetal nucleated cells are transfused following an elective first trimester termination of pregnancy. The relationship between fetal cell microchimerism and maternal disease is currently being explored. During pregnancy, fetal cells in the maternal skin are related to polymorphic eruptions of pregnancy and increased fetomaternal trafficking is detectable in cases of preeclampsia. After delivery, more male DNA of presumed fetal origin is present in the blood and skin of women with scleroderma as compared with healthy controls. Scleroderma is of particular interest because it shows a strong female predilection and it is an autoimmune disease with clinical similarities to graft-versus-host disease. Fetomaternal cell trafficking provides a potential explanation for the increased prevalence of autoimmune disorders in adult women following their childbearing years. Am. J. Med. Genet. 91:22–28, 2000. © 2000 Wiley-Liss, Inc.
Fetal Cells for Noninvasive Genetic Diagnosis

In the United States it is currently considered standard care to offer prenatal cytogenetic diagnosis via an invasive procedure such as amniocentesis or chorionic villus sampling to pregnant women who will be 35 years old or older at the time of delivery [American College of Obstetricians and Gynecology, 1996]. Although, individually, older pregnant women are at an increased risk for having a baby with trisomy 21, as a group they are responsible for a relatively small fraction of total births. Eighty percent of newborn infants with Down syndrome are born to women under age 35 who are not offered invasive procedures because the risk of a procedural complication is greater than the incidence of Down syndrome in a given fetus. Therefore, over the past decade, increased attention has been paid to noninvasive screening techniques for fetal cytogenetic abnormalities that can be offered to all pregnant women. These techniques are well documented, and include first and second trimester serum screening, and ultrasound markers such as the nuchal translucency (NT) measurement. The NT measurement is the sonographic assessment of a fluid-filled space at the back of the fetal neck. An increased NT measurement over that expected for gestational age is associated with an increased risk of fetal chromosome and cardiac abnormalities [Nicolaiades et al., 1992]. It is into the existing context of noninvasive screening for Down syndrome that research on fetal cells in maternal blood must be placed. The successful isolation of fetal cells from maternal blood represents a source of fetal chromosomes or DNA obtained noninvasively by maternal venipuncture. It is beyond the scope of the present article to give detailed information regarding this field. The reader is referred to a review that discusses this topic thoroughly [Bianchi, 1999].

The results of many years of experiments suggest that the detection of fetal cells in maternal blood has potential advantages over currently available noninvasive screening techniques. For example, approximately 50% of cases of fetal aneuploidy are detected with a false-positive rate that is significantly lower than the 5% currently used in serum screening and NT measurement [de la Cruz et al., 1998].

Our current fetal cell separation strategy is based on the isolation of fetal nucleated erythrocytes that make gamma globin. In our laboratory we use a combination of density gradient, followed by fluorescence activated cell sorting of gamma chain positive candidate fetal cells. We identify fetal cells on the basis of a characteristic morphology that includes an increased nuclear-to-cytoplasmic ratio, a brightly fluorescent stain with the antigamma globin antibody, and a positive fluorescence in situ hybridization (FISH) analysis using chromosome specific probes for X, Y, 13, 18, and 21. Our laboratory is participating in a clinical evaluation that is sponsored by the National Institutes of Child Health and Human Development, which is evaluating the accuracy of cytogenetic diagnosis using fetal cells in maternal blood, compared with the “gold standard” of a metaphase karyotype performed on amniotic fluid cells or chorionic villi [de la Cruz et al., 1995]. The results of experiments performed in our laboratory and elsewhere suggest that in a fetus with a normal karyotype, a low number of fetal cells is present in a 16–24 mL maternal blood sample [Ganshirt-Ahlert et al., 1993]. Additional studies performed using a technique known as quantitative polymerase chain reaction (qPCR) analysis have suggested that there is a mean of one fetal nucleated cell per 16 mL of maternal venous blood. Both intact fetal cells (as assayed by the FISH technique) and whole blood PCR) and fetal DNA are increased in the maternal circulation if the fetus has trisomy 21 [Bianchi et al., 1997; Lo et al., 1999a]. Currently, it is thought that this occurs because of an abnormality in the microenvironment of the placenta when the fetus has Down syndrome. Pathologic studies of placentas obtained from fetuses with Down syndrome indicate abnormalities such as villus edema in almost all placentas examined [Labbé et al., 1989]. Other abnormal fetal conditions, such as the presence of hydrops fetalis due to a developmental abnormality, such as congenital cystic adenomatoid malformation of the lung, also appear to result in increased fetomaternal transfusion.

In 1997, in collaboration with Katherine Klinger’s group at Genzyme Genetics, we developed a technique of assaying whole blood cellular DNA for the presence of fetal nucleated cells [Bianchi et al., 1997]. This study was undertaken because of preliminary evidence from sorted fetal cells indicating that relatively few fetal cells were present in most maternal blood samples [Bianchi et al., 1994]. At the time it was not known whether fetal cell loss occurred due to the isolation procedures necessary to detect fetal cells, or if there was simply a low number of fetal cells present due to intrinsic biological factors. The qPCR technique involves amplification of a sequence present on the long arm of the Y chromosome, followed by phosphorimage analysis and quantitation using male DNA standards that are run simultaneously with the experimental samples. It is important to note that both male and female fetal cells cross into the maternal circulation; however, it is easier to show unequivocally that the cell is fetal if it carries a Y chromosome. This study, performed on blood samples obtained from 220 pregnant women, demonstrated a significantly increased number of male fetal cell equivalents when the fetus was male, as opposed to female. In the fetuses with a normal male karyotype, results varied. Approximately 25% of the maternal samples contained between 0 and 5 fetal cells, 25% between 6 and 10 fetal cells, 25% between 11 and 30 fetal cells, and 25% more than 30 to 90 fetal cells. Interestingly, fetuses with trisomy 21 had an approximately sixfold-increased number of fetal cells when compared with the fetuses with a normal karyotype. These differences were statistically significant [Bianchi et al., 1997]. These data strongly suggested that the underlying fetal condition could influence fetomaternal transfusion.

The qPCR data demonstrated that a small number of intact fetal cells cross the placenta during pregnancy. We next determined the number of fetal cells that cross into the mother as a result of an elective first trimester termination of pregnancy. We obtained 40 samples
from women between 7 and 20 weeks of gestation, and found a mean of 1,528 fetal cells (range: 4 to 16,548) in 9 mL of blood [Bianchi et al., 1998]. The results were surprising in that they indicated the magnitude of the transfusion associated with a relatively early pregnancy termination. Our calculations indicated that a woman who has just undergone pregnancy termination may be exposed to a fetal cell transfusion of as many as 500,000 nucleated cells. These results have important implications when we begin to consider the persistence of fetal cells and the possibility of long-term medical complications resulting from fetomaternal transfusion.

**Fetomaternal Cell Trafficking and Pregnancy Complications**

The increased recognition of fetal cells in maternal blood and the ability to quantitate them has created an interest in potential diseases of pregnancy that might be related to fetal cells. Several studies implicate fetomaternal transfusion of both the trophoblast sprout [Sargent et al., 1994] and fetal nucleated erythrocyte [Holzgreve et al., 1998] in the development of preeclampsia. Holzgreve et al. were able to demonstrate that more fetal cells (as defined by the presence of a Y-chromosome specific probe signal) are present in pregnant women with preeclampsia, as compared with normal control women.

These data again emphasize the relationship between an abnormal placenta and increased fetomaternal transfusion. Aractingi studied women with a polymorphic skin eruption of pregnancy (PEP) [Aractingi et al., 1998]. This cutaneous eruption occurs most commonly during the third trimester of pregnancy and is characterized by the development of pruritic skin papules, plaques, and vesicles. The condition is of unknown pathogenesis and disappears after delivery. These investigators analyzed skin samples from 10 women with PEP who were carrying male fetuses and 26 pregnant women with normal skin or other skin disorders. DNA was extracted from dermis and epidermis and amplified for the presence of the SRY gene. Male DNA was detected in the skin lesions from 6 of 10 of the women with PEP but not in the other 26 pregnant women, 13 of whom were carrying male fetuses [Aractingi et al., 1998]. These results showed that fetal cells can migrate to the maternal skin during pregnancy and may be associated with a cutaneous disease.

Until recently, most investigators considered only the presence of intact fetal cells in maternal blood. In 1997, Lo et al. described a sensitive technique of real-time PCR analysis that can detect minute quantities of fetal DNA in maternal plasma [Lo et al., 1997]. It is difficult to know what correlation exists between the intact fetal cells and the degraded fetal DNA present in maternal plasma and serum. When Lo et al. [1998b] studied fetal DNA sequentially during pregnancy, they demonstrated that it accumulated in maternal serum and plasma as the pregnancy advanced. What was particularly interesting was the sharp increase over the last 8 weeks of pregnancy, which could indicate a gradual breakdown of the fetomaternal placental interface [Bianchi, 1998]. Lo et al. demonstrated that in early pregnancy (between 11 and 17 weeks of gestation) the mean concentrations of fetal DNA in maternal serum and plasma were 0.13 and 3.4%, respectively. This amount greatly increased in late pregnancy (>37 weeks), when the mean concentrations of fetal DNA detected were 1.0 and 6.2% in serum and plasma, respectively. The ability to detect such small amounts of fetal DNA in small volumes of maternal plasma has important clinical applications. In a follow-up study, Leung et al. [1998] demonstrated that fetal DNA was increased in the serum of pregnant women in preterm labor who failed tocolysis. These data suggest that placental apoptosis and breakdown has already occurred, resulting in release of fetal DNA into the maternal circulation. However, in sequential blood samples obtained from 12 women immediately postpartum and hours to 42 days after delivery of a male infant, this group also showed that the fetal DNA cleared rapidly and was completely absent from the circulation of most women within two hours of delivery [Lo et al., 1999b]. The mean half-life for the circulating fetal DNA was 16.3 minutes.

**Postpartum Persistence of Fetal Cells**

If fetal DNA is cleared rapidly after delivery, what is the fate of the fetal cells that are transfused during pregnancy, labor, and delivery? There are several lines of evidence that suggest that fetal cells can persist for decades postpartum. The existence of lymphocytes of fetal origin in maternal blood has been demonstrated using cytogenetic techniques between one and five years after the birth of a male infant [Cianfanelli et al., 1977; Schröder et al., 1974]. Using PCR amplification of Y-chromosome specific sequences in DNA extracted from blood cells of women who had previously given birth to a male infant, some investigators have found evidence of male DNA for several months after delivery; other investigators have not [Hamada et al., 1994; Hsieh et al., 1993]. It is important to recognize that these groups did not use any technique of fetal cell enrichment or separation prior to their PCR. In contrast, we flow-sorted a specific subpopulation of cells before PCR amplification, and made the discovery that fetal CD34+ or CD34+/CD38+ cells can persist in the mother for as long as 27 years postpartum [Bianchi et al., 1996]. In these experiments, we were initially using monoclonal antibody to the stem cell antigen CD34 to isolate fetal cells from pregnant women. We sorted samples from 36 pregnant women using this antibody. Of the 36 women, 22 had evidence of amplification of the Y-chromosome but only 14 of them had male infants. Six of the women who had female fetuses in the current pregnancy had previous male children. Two of the 8 women had previously undergone multiple elective terminations of pregnancy. This raised the question of possible persistence of fetal CD34+ cells from a prior pregnancy. We next analyzed a different group of women who were 30 to 65 years old. Each had a prior pregnancy history of between one and six male children who were between 6 months and 27 years old at the time of the blood sample. We used a nested PCR technique and found that six of the eight nonpregnant
women had consistent amplification of the CD34+/CD38+ lymphoid or myeloid progenitor cells. These cells were detected in women even as long as 27 years postpartum. Interestingly, the two women in whom we did not find evidence of fetal DNA were the ones whose sons were less than 1 year old at the time of blood sampling.

We next reviewed our previous results in our whole blood qPCR database to look for evidence of male DNA in the 105 pregnant women who were carrying a female fetus in the current pregnancy. In this group, 42 previously had a male child, 24 had never had a son but had had a previous miscarriage or an elective termination of pregnancy, and 29 women had no history of miscarrying a male, or elective termination of pregnancy. In 10 women no follow-up information was available on their prior pregnancy history. The results are shown in Table I, which demonstrates that there is an increase in the mean number of male fetal cells detectable in women who have had a male infant. These differences are significant by a one-way analysis of variance with a \( p = 0.02 \). The whole blood qPCR data adds credence to the hypothesis that in healthy women there is an extremely low number of residual male cells circulating from prior pregnancies. However, the results demonstrate persistence of male cells because a Y-chromosome PCR assay was used. If an assay was available that could detect a universal female fetal gene sequence, it would in all likelihood demonstrate that female fetal cells persist in equal numbers in the mother's circulation.

**Long-Term Effects of Fetal Cell Microchimerism in the Mother**

The studies described here then led to the following research questions: Does pregnancy (or labor, delivery, elective termination) establish a low grade, long-term state of microchimerism in the human female? Are the transfused fetal cells important or irrelevant in establishing tolerance to the current fetus and/or future fetuses? What implications do the transfused cells have for the future health of the mother?

Nelson speculated that fetal cell microchimerism might be involved in the pathogenesis of autoimmune disorders that predominantly affect women [Nelson, 1996, 1998]. In collaboration with Dr. Nelson’s group, we performed a study designed to evaluate the possible persistence of fetal cells in the blood of women who were no longer pregnant but were affected with scleroderma. Scleroderma is a connective tissue disorder that leads to fibrosis of skin and internal organs. It was selected as a disease to study because it is an autoimmune disorder with similarities to graft-versus-host disease. Furthermore, the disease has a strong female predilection and a sharply increased incidence in women following the childbearing years. For these reasons, it was of particular interest to test the hypothesis that fetal cell microchimerism was involved in the pathogenesis of scleroderma.

In the collaborative study with Dr. Nelson’s group, we again used the qPCR methodology to measure the number of male cell equivalents in blood samples obtained from women who had previously given birth to a son [Nelson et al., 1998]. The number of male cells detected was compared among different clinical populations. The study population consisted of 40 nonpregnant women who were 25 to 64 years old. All women had at least one son. Sixteen of the women were entirely healthy. Seventeen of the study subjects met the American College of Rheumatologist's criteria for scleroderma. Seven additional healthy women who were sisters of women with scleroderma also were studied. Among patients with scleroderma, 3 had limited and 14 had diffuse cutaneous disease. All scleroderma patients had disease affecting internal organs, most with lung involvement (>90%). The methods used for this study included the preparation of DNA from 16 mL of whole blood. A radioactive PCR was used with primers that amplify a sequence from the long arm of the Y chromosome known as 49A. The amplification products were quantitated using the phosphorimagery. The number of counts per minute in test samples was compared with a standard curve, converted to a cell number, and a statistical analysis was performed. The results of this study showed that the mean number of male fetal cell DNA equivalents was 0.38 (median = 0, range 0–2) cells per 16 mL whole blood in control women and 11.1 (median = 6, range 0–61) among scleroderma patients. The difference was highly significant (\( p = 0.0007 \)). Some scleroderma patients had concentrations of male DNA that were higher than those found in most pregnant women. Also investigated in this study was whether human leukocyte antigen (HLA)-compatibility of a child was associated with later development of scleroderma in the mother. DRB1 compatibility of a child from the mother's perspective was more common among scleroderma patients than controls but this was not essential for the apparent persistence of male DNA in maternal blood. This study concluded that low concentrations of male DNA can be detected in women decades after the birth of a son. DRB1 compatibility of a child was more common for the

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**TABLE I. QPCR Results in Pregnant Women Carrying a Female Fetus**

<table>
<thead>
<tr>
<th>Prior pregnancy history</th>
<th>Prior male child</th>
<th>No prior male; prior TAB/SAB</th>
<th>No known male no TAB/SAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>42</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>Mean number of male cells detected</td>
<td>3.3</td>
<td>2.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Minimum number male cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maximum number male cells</td>
<td>24</td>
<td>12</td>
<td>7.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.7</td>
<td>3.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*TAB, therapeutic abortion; SAB, spontaneous abortion.
scleroderma patients, therefore supporting the possibility that fetal cell microchimerism is involved in the pathogenesis of scleroderma.

The increased HLA class II compatibility also was observed by Artlett et al. [1997], who studied scleroderma patients and found that 26 of 37 (70.2%) had HLA class II alleles compatible for either their offspring or their mothers, as compared with only 9 of 42 (21%) controls. They hypothesized that in some patients, scleroderma may be a form of graft-versus-host disease caused by fetal or maternal cells that have crossed the placenta and have remained unrecognized due to class II HLA compatibility.

Artlett et al. [1998] validated the finding that Y chromosome-specific DNA sequences could be found in the blood of women with scleroderma who had previously given birth to a male child. Furthermore, they extended their observations by detecting the interphase Y chromosome as well as Y chromosome-specific sequences in the skin lesions of some women with scleroderma. Male DNA was found more often and in larger amounts in the women with scleroderma than in the normal women.

Additional information was published recently regarding the existence of mature immunocompetent cells of fetal origin in the blood of parous women. Peripheral blood mononuclear cells (PBMC) obtained from 68 parous women were analyzed for the presence of male DNA; of these, 20 had flow-sorting experiments performed using subsets of cells [Evans et al., 1999]. Fetal cell microchimerism was demonstrated in PBMC from 16/48 (33%) healthy women and 12/20 (60%) women with scleroderma. Microchimerism was found in some women in CD3+, CD14+, CD19+, and CD56+/CD16+ subsets up to 38 years after pregnancy. In this study, HLA compatibility between a mother and child was not required to detect persisting fetal cells. The authors concluded that specific immunoregulatory pathways must exist that allow fetal cells to persist but prevent their immune function in healthy women [Evans et al., 1999].

To date, the association between fetomaternal cell trafficking and diseases in parous women has been shown for preeclampsia and the development of PEP during pregnancy, whereas scleroderma becomes an issue postpartum. The hypothesis that fetal cells can be involved in inflammatory or autoimmune disorders that predominantly affect women can now be tested in a variety of other conditions (Fig. 1). Other candidate diseases that merit attention include primary biliary cirrhosis, thyroiditis, lupus, rheumatoid arthritis, and primary pulmonary hypertension.

Thus far, we have focused on the isolation of fetal cells in maternal blood for the purpose of noninvasive genetic analysis. Now that we know that fetal cells are in the mother, we must ask why are they there? Where do they migrate in the mother’s body, and what do they do to her microenvironment? What implications do these cells have for the long-term health of the woman who has undergone pregnancy?

![Feto-Maternal Cell Trafficking: Fetal Cells in Mother](image)

- Pre-eclampsia
- Scleroderma
- Polymorphic eruptions of pregnancy
- Primary biliary cirrhosis?
- Thyroid disease?
- Lupus?
- Rheumatoid arthritis?
- Primary pulmonary hypertension?
- Sjögren syndrome?

Fig. 1. Diseases potentially associated with fetal cell microchimerism. Evidence exists for the conditions above the arrow that fetal cells are involved. The role of fetal cells in the conditions listed below the arrow is speculative at present.
Maternal Cells in the Fetus

An important corollary to the above questions is: do maternal cells get into the fetal circulation and what do they do there? This question is of particular concern because of the storage of umbilical cord blood, which may contain contaminating immunocompetent maternal cells. A variety of studies have identified the presence of maternal cells in the fetus. Using a PCR-based minisatellite assay, Socié et al. [1994] detected maternal cells in umbilical cord blood samples in 1 of 47 cases. Using a FISH assay with X- and Y-chromosome specific probes Hall et al. [1995] detected maternal cells in 10 of 49 male umbilical cord blood specimens. Using a more sensitive assay for β globin polymorphisms, Lo et al. [1996] found evidence of maternal cells in 16 of 38 cord blood samples. Antenatal transfer of maternal cells into the fetus occurs as early as 13 weeks of gestation [Lo et al., 1998a]. The possible transfusion of maternal cells into the fetus may have a role in the development of inflammatory and autoimmune disorders that affect the infant, child, and adult.

This hypothesis has been tested in a study that developed sensitive HLA-specific PCR assays to detect the presence of maternal genes not shared with the child as a means of identifying maternal cell microchimerism [Maloney et al., 1999]. In six of nine adult scleroderma patients nonshared maternal-specific DNA sequences were found in peripheral blood samples. Maternal microchimerism also was detected frequently in healthy control individuals. The results of this study demonstrated that HLA-disparate maternal cells can persist in immunocompetent offspring into adult life. The biological significance of maternal cell microchimerism in the child is presently unknown.

SUMMARY

Fetomaternal trafficking studies began with the isolation of nucleated erythrocytes from the blood of pregnant women for noninvasive genetic diagnosis. Nucleated erythrocytes have a finite life span and there is no evidence to indicate that they persist into future pregnancies; they are currently the target cell of choice for noninvasive prenatal diagnosis. The long-term persistence of fetal cells with proliferative potential should have no implications for error in genetic diagnosis in a future pregnancy as long as they are not used as a target cell. Maternal whole blood and plasma can both be used as sources of cellular and free fetal DNA. If maternal blood is used to prepare cellular DNA, assays will detect all fetal cell types, as well as prior transfusions, and potentially, cells from past and present pregnancies. Plasma fetal DNA, in contrast, is limited to the current pregnancy, and there is no evidence that this free fetal DNA persists after delivery. The long-term persistence of fetal progenitor cells and lymphocytes creates a microchimeric state in the woman who previously was pregnant. An appreciation of this phenomenon is increasing gradually. Further studies are needed to define the particular fetal cell types that are involved in microchimerism, why they can persist in the mother for many decades, and whether they are immunocompetent and capable of eliciting a graft-versus-host response.

REFERENCES


