Microchimerism

An Investigative Frontier in Autoimmunity and Transplantation

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Recent studies indicate cells transfer between fetus and mother during pregnancy and can persist in both decades later. The presence within one individual of a small population of cells from another genetically distinct individual is referred to as microchimerism. Naturally acquired microchimerism has recently been investigated in autoimmune diseases, including scleroderma, thyroiditis, primary biliary cirrhosis, Sjögren syndrome, systemic lupus, dermatomyositis, and neonatal lupus. Iatrogenic chimerism has been investigated in transplantation and following blood transfusion. Considering findings of naturally acquired microchimerism along with iatrogenic microchimerism suggests microchimerism can have detrimental and/or beneficial effects in both settings. Recent identification of tissue-specific microchimerism either from naturally acquired or iatrogenic microchimerism (eg, cardiac myocytes) raises the possibility that microchimerism can be a target of autoimmunity or alternatively contribute to tissue repair. Advances in this new frontier of research with varied and numerous implications for human health are summarized.

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MICROCHIMERISM REFERS TO a small population of cells or DNA in one individual that derives from another genetically distinct individual. Cell traffic between mother and fetus during pregnancy has recently been found to result in long-term persistence of fetal cells (fetal microchimerism) in the mother and maternal cells in her progeny (maternal microchimerism). Microchimerism may also result from twin-twin transfer in utero. Although not formally proven, fetal microchimerism is presumed to persist after miscarriage and abortion. Theoretically, microchimerism could also derive from an older sibling transferred via the maternal circulation to the fetus of a later pregnancy.

Recent studies have investigated a potential role of naturally acquired fetal and maternal microchimerism in autoimmune diseases, including systemic sclerosis (scleroderma), thyroiditis, primary biliary cirrhosis (PBC), Sjögren syndrome, systemic lupus erythematosus (SLE), dermatomyositis, and neonatal lupus syndrome. While lending support to the concept that microchimerism may contribute to some autoimmune diseases, studies have also shown that naturally acquired fetal and maternal microchimerism are common in healthy individuals.

Whereas the appreciation of naturally acquired microchimerism is relatively new, a more extensive literature has examined iatrogenic chimerism in hematopoietic cell transplantation (HCT), organ transplantation, and following blood transfusion. Iatrogenic chimerism in HCT can result in chronic graft-vs-host disease, a disorder with striking clinical similarities to autoimmune disease, including development of autoantibodies. The recent finding that healthy individuals often have low levels of maternal microchimerism may have relevance to prior studies in transplantation describing tolerance to organs mismatched for noninherited maternal HLA antigens. Microchimeric cells expressing tissue-specific markers have recently been reported in transplantation as well as in autoimmune disease, suggesting microchimeric cells may differentiate and thus could contribute to tissue repair or be a target in autoimmune disease. Recent findings in naturally acquired microchimerism reveal areas of common insight with iatrogenic microchimerism and suggest that detrimental and beneficial effects occur.

See also p 1114.

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Methods of Detecting Microchimerism

All but a few studies have used identification of male DNA or male cells in a woman as the demonstration of microchimerism. This approach is used for the practical reason that 1 assay can test

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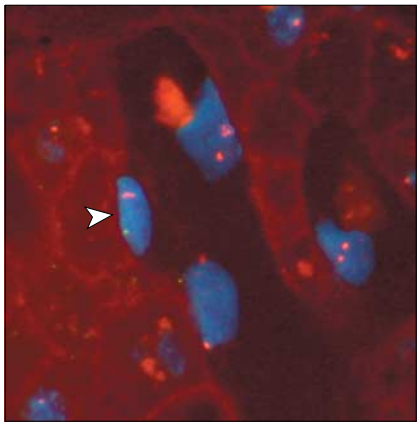
Figure 1. Male Cell in Female Liver by Fluorescence In Situ Hybridization



White arrowhead indicates a male cell containing 1 Y chromosome (green) and 1 X chromosome (red) surrounded by female cells containing 2 X chromosomes in the liver specimen of a woman with systemic sclerosis (scleroderma) (magnification ×100). The Y chromosome–specific probe for DYZ1 was labeled with green fluorescent fluorescein-isothiocyanate–linked deoxyuridine 5-triphosphate. The X chromosome–specific probe for DXZ1 (centromere-associated) was labeled with red fluorescent cyanine-3–linked deoxyuridine 5-triphosphate. Nuclei are identified by a blue nuclear counterstain, 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI) and analyzed by fluorescence microscopy with a triple-band filter specific for fluorescein, cyanin 3, and DAPI.

many individuals. Results represent presumptive evidence of fetal microchimerism in women with prior male pregnancies or, in transplantation, of male donor microchimerism in female recipients. Male DNA is detected by polymerase chain reaction (PCR) amplification of a Y chromosome–specific sequence. Male cells are detected by fluorescence in situ hybridization with labeling of the X and Y chromosomes. FIGURE 1 is an example of this approach in which a male cell can be observed among female cells because a fluorescent green probe identifies the Y chromosome while a fluorescent red probe identifies the X chromosome. The fluorescence in situ hybridization technique can also be used to identify maternal microchimerism in a man. Male cells could derive from an unrecognized twin, possibly from an older male sibling, and in transplantation studies from prior pregnancies of the donor or recipient. Many studies use nonquantitative PCR techniques and report results in terms of the frequency of microchimerism (ie, any positive result in cases vs controls). A more powerful approach is to use quantitative methods and report levels of microchimerism, an approach that is further enhanced by targeting nonshared genetic polymorphisms to better define the source of microchimerism.[1] Interpretation of studies requires consideration of the technique used, especially important because microchimerism is a low-frequency event and standardization of methods has been lacking. Study design is also important as is inclusion of a woman's pregnancy history when male DNA is used to identify microchimerism.

Fetal Microchimerism in Autoimmune Diseases

The first study that investigated naturally acquired microchimerism in an autoimmune disease was a prospective blinded study of fetal microchimerism in women with systemic sclerosis.[2] The study was initiated based on observations including clinical similarities between systemic sclerosis and chronic graft-vs-host disease, increased systemic sclerosis incidence in women after reproductive years, and appreciation of an important role for HLA genes in autoimmunity and chronic graft-vs-host disease.[3] A quantitative assay was used to test for male DNA in peripheral blood from women with systemic sclerosis and healthy women who had previously given birth to at least 1 son. Levels of male DNA were significantly higher in women with systemic sclerosis compared with healthy women (mean male DNA cell equivalent per 16 mL of whole blood: 11 vs 0.4, respectively). Some women with systemic sclerosis who had borne a son decades previously had results as high as the highest quartile of results using the same assay to test women currently pregnant with a healthy male fetus. This study also reported results of DNA-based HLA typing in which women with systemic sclerosis, healthy women, and all their children were studied. Prior birth of a child indistinguishable from the mother for genes encoding HLA-DR molecules was associated with more than 7-fold increased risk of systemic sclerosis in the mother. Considered together the findings of increased levels of fetal microchimerism and an increase of HLA-DR compatibility support a potential role in disease pathogenesis.

Two subsequent studies tested for male DNA in lesional skin of women with systemic sclerosis, both with positive results.[4,5] The former used a nonquantitative technique and described an increased frequency of fetal microchimerism whereas the latter used a quantitative technique and found no difference in frequency but significantly higher quantities of male DNA in women with systemic sclerosis vs controls (4.6 vs 1.8 male DNA cell equivalent per 80 ng of tissue, respectively). In another study of autopsies from 5 women with systemic sclerosis,[6] male cells were found in lung, kidney, skin (systemic sclerosis disease sites), liver, adrenal, and lymph nodes of some patients with especially high numbers in spleen. All patients with systemic sclerosis had given birth to sons. No male cells were found in limited sampling of tissues from 3 controls, 2 of whom had given birth to sons. Splenic fetal microchimerism was of interest in light of an experimental model in which mice treated with vinyl chloride, an agent associated with systemic sclerosis, had levels of fetal microchimerism that correlated with dermal fibrosis and exhibited marked splenomegaly.[7]

Overall, studies in systemic sclerosis may be summarized as showing a significant difference in fetal microchimerism quantity in blood and tissues vs controls without necessarily showing an increased frequency, although additional studies, especially with comparison to nonautoimmune inflammatory conditions, are needed.[8] It may be argued that elevated levels of fetal microchimerism in blood and disease-affected tissues could occur secondary to inflammation. However, the finding of HLA-compatibility as a risk factor lends support to a role of microchimerism in disease pathogenesis.[2]

One possibility is that microchimeric cells are direct effectors, as suggested by a study in which male T-cell clones from blood and skin of women with systemic sclerosis reacted against maternal HLA antigens.[9] However, because the number of microchimeric cells is very low, far less than 1% in peripheral blood, other mechanisms seem likely. For example, immune responses might be amplified by indirect antigen presentation, a mechanism thought to be involved in chronic organ rejection.[8]

Studies of fetal microchimerism in other autoimmune diseases have yielded more variable results. Autoimmune thyroid disease is of interest because of a marked female excess and especially high postpartum incidence. An increased frequency of male microchimerism (presumed fetal) in female thyroid tissue has been described for patients with Hashimoto disease[10] and Graves disease vs controls,[11] although male cells were almost as common in some other nonautoimmune thyroid conditions in another study.[12] Primary biliary cirrhosis, another autoimmune disease with a marked female predilection, is of additional interest in that it bears pathological resemblance to graft-vs-host disease of the liver. All but 1 study found no significant difference in frequency of male microchimerism in female PBC vs control liver biopsies, although levels were somewhat higher in PBC.[8] The frequency of positive results varied over a wide range (18%-70%) that is best explained by differences in patient selection (some included women without sons) and by differences in technique. Overall, current results suggest fetal microchimerism does not play a major role or is not a sufficient risk factor for PBC. However, no study has yet considered whether immunologic consequences of fetal microchimerism might differ depending on pregnancy type (eg, miscarriage or induced abortion) or number of partners. A recent study described an increasing risk of PBC with increasing gravidity and twice as many patients with PBC vs controls reporting 5 or more pregnancies.[13]

A few studies have investigated fetal microchimerism in Sjögren syndrome and SLE.[8] Attempts to identify male DNA in the salivary glands of women with Sjögren syndrome have yielded inconsistent results. The differences may be due to technique or because there are disease subsets, as Sjögren syndrome may occur secondary to another autoimmune disease or as a primary condition. In SLE, no difference of fetal microchimerism frequency was found compared with controls, although higher levels were found in patients with nephritis. Fetal microchimerism has been described in a few other case reports.

Maternal Microchimerism in Autoimmune Diseases

Maternal microchimerism has been investigated in systemic sclerosis, dermatomyositis, and neonatal lupus. Although maternal cells are known to engraft in immunodeficient infants, persistence of maternal microchimerism in immunocompetent individuals was not examined until recently. Using a dual approach targeting noninherited, nonshared maternal-specific HLA sequences and corroboration with identification of female cells in male patients by fluorescence in situ hybridization, maternal microchimerism was found to persist into adult life.[14] A later study described the development of a panel of assays that quantify maternal microchimerism by real-time PCR, targeting noninherited, nonshared specific HLA sequences.[15] Maternal microchimerism was significantly more frequent in patients with systemic sclerosis vs controls (72% vs 22%, respectively), although levels of maternal microchimerism did not differ significantly in patients with systemic sclerosis vs controls.

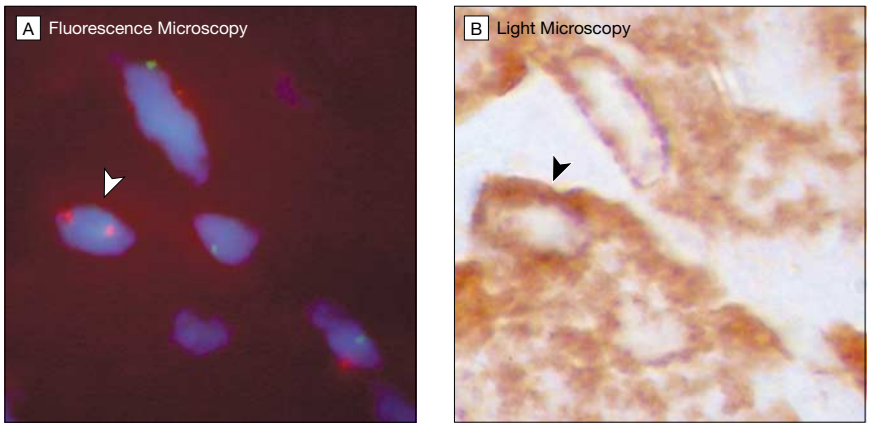
Maternal microchimerism has been examined in peripheral blood and muscle biopsies from children with dermatomyositis. The frequency of maternal microchimerism was significantly increased in blood and muscle biopsies compared with unrelated controls and unaffected siblings.[16] Inclusion of unaffected siblings as controls is a study design strength as environmental and to some extent genetic background are similar. The phenotype of maternal cells in muscle tissues was not determined in these studies.

A recent study described identification and characterization of maternal microchimerism in the heart of male infants with neonatal lupus syndrome who died from congenital heart block.[17] Maternal cells were found in the atrioventricular node and in the myocardium (up to 2% in cases vs 0.1% in controls of all cells in some sections). To characterize cells in tissue sections, a technique was developed that combined immunohistochemistry with concomitant fluorescence in situ hybridization for X and Y chromosome markers in the same cells. Surprisingly, more than 80% of the maternal cells in infant heart tissue did not express hematopoietic markers but instead expressed myocyte-specific markers (FIGURE 2). This observation suggests tissue-specific microchimerism could become the target for an autoimmune response. Alternatively, maternal cells may migrate to areas of tissue damage secondarily and function beneficially in repair.

Transplantation and Transfusion From the Perspective of Naturally Acquired Microchimerism

Although long-term persistence of naturally acquired fetal and maternal microchimerism has been appreciated only recently, chimerism in transplantation has been a subject of investigation for more than a half century. In organ transplantation, recipient microchimerism with donor cells occurs and has been proposed as a mechanism facilitating graft acceptance.[18] The relationship of donor microchimerism to graft acceptance, however, has been controversial. Because most studies of donor microchimerism in organ transplantation were performed prior to the recent knowledge that naturally acquired fetal and maternal microchimerism are common, new studies that consider confounding with naturally ac-

Figure 2. Maternal Cell With a Myocardial Tissue–Specific Phenotype in a Male Infant Heart by Fluorescence In Situ Hybridization and Concomitant Immunohistochemistry



A, Fluorescence microscopy, white arrowhead indicates a female cell (presumed of maternal origin) with 2 X chromosomes (red fluorescent dye) observed among male cells with 1 Y chromosome (green fluorescent dye) in the heart tissue of a male infant who died from congenital heart block associated with neonatal lupus syndrome (magnification ×100). The Y chromosome–specific probe for DYZ1 was labeled with green fluorescent fluorescein-isothiocyanate–linked deoxyuridine 5-triphosphate. The X chromosome–specific probe for DXZ1 (centromere-associated) was labeled with red fluorescent cyanine-3–linked deoxyuridine 5-triphosphate. Nuclei are identified by a blue nuclear counterstain, 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI) and analyzed by fluorescence microscopy with a triple-band filter specific for fluorescein, cyanin 3, and DAPI. B, Light microscopy, black arrowhead indicates the same maternal cell as in A. The brown staining within the cytoplasm surrounding the female nucleus indicates sarcomeric α-actin expression, consistent with a cardiac myocyte phenotype. Immunohistochemistry was performed with antibodies to sarcomeric α-actin, a peroxidase development system, and developed with diaminobenzene (magnification ×100). Printed with permission from Anne Stevens, MD, PhD.

quired microchimerism and that use quantitative techniques specific only for donor microchimerism may help clarify the role of donor microchimerism in allograft survival. The concept that microchimerism can affect transplantation tolerance appeals for analogy to naturally acquired fetal microchimerism in pregnancy biology in which mechanisms must exist to promote fetal tolerance to prevent rejection based on genetic differences.

Studies in transplantation have described recipient tolerance to noninherited maternal HLA antigens. Although not inherited, maternal HLA antigens can be acquired as microchimerism,[15] affording a new perspective on findings in transplantation. In a multicenter analysis of kidney transplants involving sibling donors mismatched for 1 HLA haplotype, significantly enhanced graft survival was observed when the donor had the recipient's noninherited maternal HLA haplotype.[19] In HCT with related donors, lower acute graft-vs-host disease rates were found with sibling donors mismatched for noninherited maternal HLA antigens.[20] A recent small study suggested mismatched HCT without T-cell depletion might be feasible by considering tolerance to naturally acquired microchimerism.[21]

Blood transfusion has been investigated as a means for inducing donor-specific tolerance before organ transplantation. Among patients awaiting first renal transplant, blood transfusion induced tolerance to HLA antigens of the transfusion donor, particularly when there was a common HLA haplotype or shared HLA-B and HLA-DR antigens.[22] Similar to studies of naturally acquired microchimerism, long-lasting donor microchimerism has been reported after blood transfusion in multiply transfused trauma patients.[23]

Other studies illustrate settings in which naturally acquired microchimerism may have adverse consequences in transplantation. For example, when a woman is a donor for HCT, is fetal microchimerism also transferred? A female donor may be tolerant of her fetal microchimerism but tolerance cannot be presumed in the recipient, particularly after undergoing immunosuppression. Lending support to this possibility, male DNA was identified in 34% of growth factor mobilized peripheral blood cells and 48% of CD34-enriched apheresis female donor products.[24] This could represent 10 000 to 40 000 male cells infused during a typical HCT, estimating from the lowest quantity of male DNA detected in the study. Although other factors such as minor HLA antigens are known to play a role in graft-vs-host disease observed in HLA-identical sibling HCT,[25] fetal microchimerism of the donor or the recipient could in part explain graft-vs-host disease in HLA-identical sibling and identical twin HCT, as well as increased graft-vs-host disease risk with parous female donors.

An adverse effect of pregnancy has also been suggested in kidney transplantation with spousal donors with somewhat better graft survival for women recipients who had not been pregnant compared with those who had been pregnant by the spouse.[26] That immunologic effects of fetal microchimerism might differ from that of maternal microchimerism in the setting of organ transplantation is not unexpected, particularly because exposure to fetal microchimerism occurs in adult life whereas exposure to maternal microchimerism occurs during fetal life while the immune system is still developing.

Conclusions

Recent knowledge that fetal and maternal microchimerism is a common sequelae of pregnancy invites questioning of the traditional view of autoimmunity and alloimmunity. Some studies have provided support for the concept that naturally acquired microchimerism can contribute to autoimmune diseases but at the same time suggest microchimerism may have beneficial effects to the host. It is likely that fetal and maternal microchimerism can

have adverse, neutral, or beneficial effects on the host, depending on other factors such as specific HLA genes and the HLA relationship between cell populations. Elucidating the mechanisms by which naturally acquired microchimerism occurs without detriment to the host may lead to novel strategies for treatment of some autoimmune diseases and potentially to improvement in morbidity and mortality associated with transplantation.

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Intelligence is characterized by a natural incomprehension of life.
—Henri Bergson (1859-1941)