ta from properly remodeling or make it more likely to admit excess cortisol.

Sculpting destiny

If the 9 months spent in the womb help shape susceptibility to disease, what, if anything, can be done to reverse or even prevent ill effects? Given how little is known about critical periods in pregnancy when a given stressor might produce a given defect, the likelihood of preventing problems before they start remains remote.

Reversing these problems could prove slightly more feasible. One possibility might be halting a common phenomenon among babies born smaller than intended, called catch-up growth. These infants born at, say, the 20th percentile for growth hit the 80th percentile by the time they're school age. Some theorize that this occurs because a baby conditioned in the womb to anticipate fewer nutrients gains more from each gram of food it consumes. Studies in humans and animals suggest that catch-up growth makes adult diseases associated with low birth weight much likelier. Bagby is experimenting in her pigs to see whether inhibiting catch-up growth—which, in humans, might be easier said than done—will preserve kidney function and normal blood pressure in adults.

In another approach, treating newborn rats between 2 and 4 weeks after birth with drugs to counteract high blood pressure, and then withdrawing the treatment, has also been shown to permanently reverse the effects of low protein prior to birth, says Langley-Evans.

Still, researchers say they're a long way from addressing the implications of troubled fetal environments in the clinic, especially because low birth weight remains the only simple measure of susceptibility to later disease. Rebecca Simmons, a neonatologist at the University of Pennsylvania and Children's Hospital of Philadelphia, routinely treats low–birth-weight babies. But at a loss to quantify risks, she rarely volunteers information on the likelihood of later disease with parents. "The reason we're not talking about it with the parents now," she says, "is that we don't know what to do."

–JENNIFER COUZIN

NEWS

Cells Exchanged During Pregnancy Live On

Microchimerism, viewed at first as an oddity, has been linked to autoimmune diseases and complications of pregnancy

A mother's love is enduring. But most mothers would be surprised to discover that there's a similarly enduring physical bond: Cells from a fetus can live on in the mother's body for decades after pregnancy, a situation called microchimerism. Likewise, a mother's cells can also survive for many years in her child.

When this phenomenon was first reported in the mid-1990s, scientists scoffed at the notion that these cells could persist for so long, tolerated by their host's immune system. "Everyone said it can't be true," says rheumatologist Michael Lockshin, director of the Barbara Volcker Center for Women and Rheumatic Disease at the Hospital for Special Surgery in New York City. "But now everyone who looks finds it."

In some cases, the cells might be benign guests: self-perpetuating lines of stem cells that can reproduce and even give rise to other types of cells, all without harming their host. But a growing body of research, still preliminary, suggests that the cells might also be at the root of some autoimmune diseases and other conditions.

Indeed, microchimerism might help explain one of the puzzles about autoimmune diseases: why many of them strike more women than men. No one knows how many women carry foreign cells around from past pregnancies, but several studies have shown that women with certain autoimmune diseases are more likely to harbor such cells than healthy women. "When you see that this is a real phenomenon, it gives you a different perspective," says pediatric hematologist William Reed of the Children's Hospital Research Institute in Oakland, California. "You begin to ask yourself whether a disease might have a pathogenesis that you've never considered before."

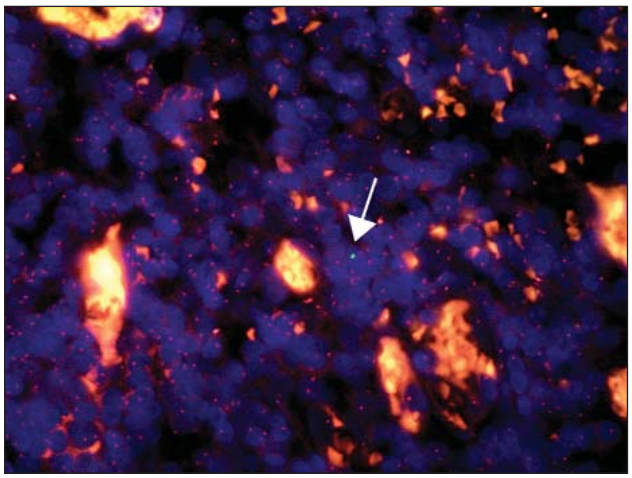
And it's not only the long-lived cells that might be making mischief. Reproductive biologists have known for some time that fetal cells course through the bloodstream of pregnant women, but in the past 4 years researchers have discovered that this temporary invasion might be implicated in two common complications of pregnancy.

Inner turmoil

Fetal microchimerism was uncovered quite by chance. In 1992, medical geneticist Diana Bianchi, then at Children's Hospital in Boston, was trying to develop a method for prenatal diagnosis that relied on isolating fetal cells from the blood of pregnant women. Her team was separating out cells that carried a protein known as CD34—a marker for so-called hematopoietic stem cells that give rise to cells of the immune system—based on a hunch that CD34 would be a good marker for fetal cells.

Blood from 13 of the pregnant women they studied contained CD34-positive cells with a Y chromosome, indicating that the fetuses from which the cells came were male. But amniocentesis showed that only nine of those women were carrying male fetuses. "We were mystified," says Bianchi, who is now at Tufts–New England Medical Center in Boston. They checked to see whether any of the four women with unexplained male cells had other children who were male, and two of them did. The other two had previously terminated pregnancies in which the sex of the fetus was not known. "That is when the hypothesis began to take shape," says Bianchi.

To test the idea that fetal cells from a past pregnancy can linger, Bianchi and her colleagues examined the blood of mothers who were not pregnant. They chose eight mothers of boys, because testing for the presence of a Y chromosome could easily distinguish the sons' cells from their mothers'. Six of the women, including one whose youngest son was 27 years old, had male cells still circulating in their blood. The idea was so surprising that it met resistance. "I lost count of how many times this paper was rejected," Bianchi told the



Under mom's skin. A cell with a green-stained Y chromosome, presumably from a son, was found in a skin biopsy from a woman with systematic sclerosis.

CREDIT: KIRBY L. JOHNSON/TUFTS-NEW ENGLAND MEDICAL CENTER

audience at a meeting in April.* The study was finally published in the *Proceedings of the National Academy of Sciences* in January 1996.

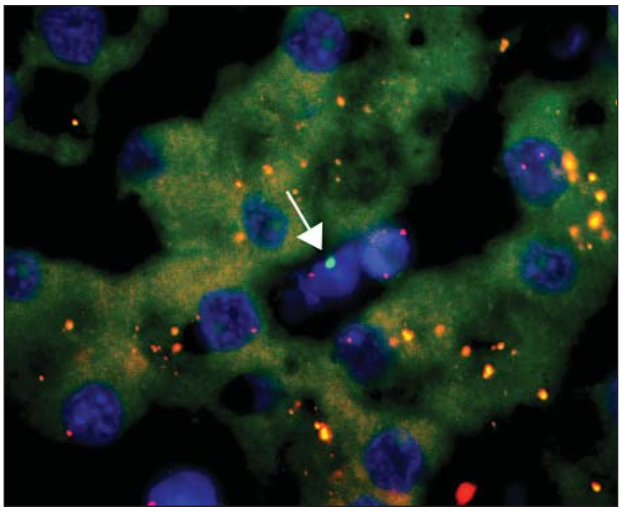
Meanwhile, on the other side of the country, Lee Nelson, an immunologist and rheumatologist at the Fred Hutchinson Cancer Research Center in Seattle, had formulated a theory that fetal cells lingering in the mother might be at the root of autoimmune disease. Nelson studies autoimmune diseases such as scleroderma, a debilitating condition characterized by inflammation of the skin, which is often called systemic sclerosis when it advances to involve internal organs. The symptoms resemble graft-versus-host disease (GVHD), a complication that sometimes arises in bone-marrow-transplant recipients, when white blood cells derived from the donated bone marrow attack the recipient's tissues. Because scleroderma, like many autoimmune diseases, is more common in women than in men and often arises after a woman's childbearing years, Nelson wondered whether it might be caused by an immune reaction set off by pregnancy.

In 1994, before seeing Bianchi's work, Nelson heard from a colleague that researchers at CellPro, a Seattle biotech company, had found fetal cells in a woman years after pregnancy. Although that work was never published, Nelson contacted Jeff Hall, the CellPro scientist who had made the observation, and Hall told her about Bianchi's work. Nelson wrote a paper outlining her hypothesis that fetal cells might trigger autoimmune disease, which was published in *Arthritis & Rheumatism* in February 1996.

Nelson contacted Bianchi, and the two began collaborating to search for fetal cells in the blood of female scleroderma patients. They tested 17 patients and 23 healthy women, all of whom had given birth to at least one son. They found male (presumably fetal) cells in most of the patients and in some of the healthy controls. But overall, the scleroderma patients had 30 times as many fetal cells in their blood as the healthy women did, an average of seven male cells per 10 milliliters of blood. The foreign cells they identified were cells from the fetal immune system, including antibody-producing B cells and T cells—the type of cell that is responsible for the cellular immune response that contributes to scleroderma—as well as natural killer cells and monocytes. In short, circulating in the women's blood were cells that were tuned to protect the fetus against foreign invaders and that could conceivably recognize the women's own tissues as foreign and attack them.

The researchers also found evidence that, just as in GVHD, the compatibility between the foreign cells and their host seems to play a role. The histocompatibility, or *HLA*, genes encode proteins that help the immune system identify and kill cells that have foreign proteins on their surface, such as virus-infected cells. Each *HLA* gene comes in up to 100 different forms, and immune cells will also kill cells that have forms of the HLA proteins that are different from their own. This is why *HLA* genes must be carefully matched between bone marrow donors and recipients.

Each person has two copies of each *HLA* gene, one from each parent, so a mother has one copy in common with her child. The child's other copy, which comes from the father, is usually different. In women with scleroderma, however, Nelson found that for one of the *HLA* genes, known as *DRB1*, both copies of the gene carried by the fetal cells matched their mother's genes. The fetus either had the same two genes that the mother did, or the fetus had two identical copies that matched one of the mother's genes. In either case, the mother's immune system would not recognize that fetal *HLA* gene as foreign.



Persistence. A cell with a green-stained Y chromosome in a mother's liver biopsy suggests that fetal cells endure for decades.

But the so-called HLA compatibility for the *DRB1* gene alone can't explain why the mother's immune system doesn't kill the fetal cells, Nelson says. The other *HLA* genes are unlikely to be matched between fetus and mother, and those mismatches should cause the mother's immune system to destroy the fetal cells. "Why these cells persist in the face of all these mismatched *HLA*s is a very interesting biological question," she says.

The fetal cells do persist, though, and their HLA compatibility with the mother for the *DRB1* gene is associated with an increased risk of scleroderma. Nelson's team calculated that a woman who has given birth to a child whose *DRB1* genes each match one or both of hers has a ninefold greater risk than average of developing scleroderma.

If these fetal cells are somehow mounting an attack on the mother's tissues, one would expect to find fetal cells at the site of inflammation. In 1998, Carol Artlett, Sergio Jimenez, and their colleagues at Thomas Jefferson University Hospital in Philadelphia found this to be the case: They identified male cells in the skin lesions of 11 out of 19 women with scleroderma.

Meanwhile, other researchers looked for fetal cells in patients with other autoimmune diseases. Two groups, Bianchi's at Tufts and that of Michael Klintschar at Martin Luther University in Halle-Wittenberg, Germany, found increased numbers of fetal cells associated with Hashimoto's thyroiditis, an autoimmune disease that reduces the patient's production of thyroid hormone and strikes more women than men.

With child, with cells

Although researchers were surprised to find fetal cells persisting in the mother for years after birth, it has long been known that fetal cells enter the mother's blood during pregnancy. Now researchers suspect that these cells are at work in at least two diseases of pregnancy—one an autoimmune disease, the other probably not.

Dermatologist Selim Aractingi of Tenon Hospital in Paris studies skin conditions in pregnant women, including polymorphic eruption of pregnancy (PEP), which resembles a bad case of hives. In 1998, Aractingi and his colleagues reported finding male cells in the skin lesions of five out of 10 women with PEP who were carrying male fetuses. The presence of the male cells seemed specifically associated with PEP, because the researchers found no male cells in comparable skin samples routinely removed during caesarian sections from 13 women who were delivering boys but did not have the disease.

That same year, Wolfgang Holzgreve, an obstetrician and geneticist at the University of Basel, Switzerland, was working—as Bianchi had been in Boston—on developing a way to isolate fetal cells from maternal blood for prenatal diagnosis. In the course of that study, his team made an unexpected discovery: Women with a serious complication of pregnancy called preeclampsia had manyfold more circulating fetal cells than healthy pregnant women had. "In normal pregnancy, the level of fetal cells is about one in 1 million cells in maternal circula-

CREDIT: KIRBY L. JOHNSON/TUFTS-NEW ENGLAND MEDICAL CENTER

* The Society for Women's Health Research third annual conference on Sex and Gene Expression, San Jose, California, 4–7 April.

tion," Holzgreve says. "In preeclampsia, it could be one in 1000 or more."

Preeclampsia, which causes dangerously high blood pressure, impaired kidney function, and edema, usually occurs in the third trimester of pregnancy and often forces an immediate delivery of the child to save the mother's life. The health and survival of babies born this way would be improved if physicians could prepare them in advance for a premature birth—for example, by administering treatments to accelerate lung maturation. Holzgreve wondered if the high numbers of fetal cells could be used as a predictor of preeclampsia. Together with his co-worker Sinuhe Hahn, he chose a group of women who, because of placental abnormalities detected by ultrasound, were thought to be at increased risk of preeclampsia. At the 20th week of pregnancy, the researchers drew blood from the women and analyzed it for fetal DNA. "There was a strong correlation between the level of fetal DNA and the likelihood of developing preeclampsia," Holzgreve says.

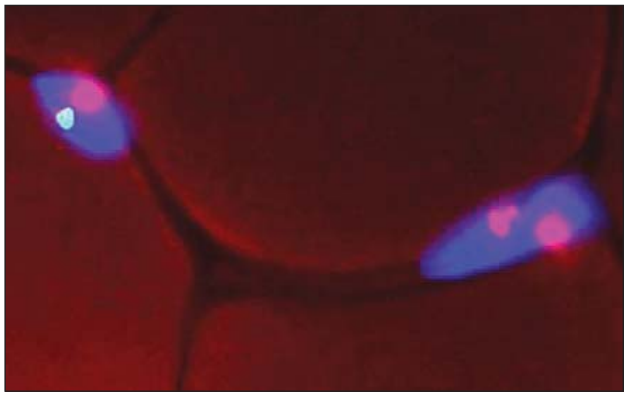
Holzgreve's team also found a parallel between the amount of fetal DNA in the mother's blood and the severity of the disease. Researchers have long suspected that in preeclampsia, some toxin in the blood damages endothelial cells lining organs such as the kidneys. Now Holzgreve suspects that the fetal cells or free fetal DNA are that toxin. Preliminary studies with endothelial cell cultures suggest that fetal cells and DNA are toxic to endothelial tissue, he says: "It could be a very direct effect of the [fetal] material on the maternal tissue."

Two-way traffic

Mothers might bear the brunt of this newly discovered cellular invasion, but it turns out that men and women who have not borne children are not exempt. Cells of close relations can colonize their kin in two other ways: Twins share cells in the womb and can harbor these cells into adulthood; and a mother's cells can stick around in her child's bloodstream for years.

Maternal cells have been linked to at least one autoimmune disease. Two research teams, one led by Artlett of Thomas Jefferson University and the other by pediatric rheumatologist Ann Reed at the Mayo Clinic in Rochester, Minnesota, independently found maternal cells in the inflamed muscle tissue of children and young adults with autoimmune conditions that attack the muscles. Reed's group studied children with juvenile dermatomyositis, and Artlett's group looked at boys with either dermatomyositis or a related condition called polymyositis. The researchers found maternal cells in the bloodstream and tissues of nearly all of the boys they examined who had the autoimmune conditions, compared with 20% or fewer of those who did not. And when maternal cells were present in healthy boys, they were generally in lower abundance.

Like Nelson's group, Reed's team also found a striking association between disease and certain HLA relations. In this case, it wasn't a simple matter of the son's and mother's *HLA* genes matching. Rather, 85% to 90% of the patients had a particular version of an *HLA* gene known as *DQ*. The gene doesn't always cause trouble, but it might somehow enable maternal cells to persist in children. What's more, Reed's team found that the children without dermatomyositis who had maternal cells in their blood carried the same form of the *DQ* gene as did the boys with the disease. "The gene seemed to influence somehow the persistence of these cells in the children," says Reed.



Inheritance tax. A boy with juvenile dermatomyositis hosts a blue-stained white blood cell (right) with two X chromosomes (pink) and no Y chromosomes (green).

That might be the key to explaining another nagging puzzle in autoimmunity research, Reed suggests. For some reason, people with certain HLA types are more likely to suffer from some autoimmune diseases. "Maybe it is not the *HLA* gene per se causing autoimmunity," she posits. "Maybe it is allowing chimerism to occur and then that is triggering the disease." Her current hypothesis for dermatomyositis, she says, is that there is a mutual tolerance between the patient and his mother's cells, perhaps enabled by the patient's *DQ* gene. Then some second event causes the tolerance on the part of the mother's cells to break down, and the mother's cells attack the patient's tissues.

Both teams found evidence that stem cells from the mother have set up housekeeping in the patients' bone marrow—a sort of mini–bone marrow transplant. Maternal cells in the patients include T cells and B cells, which could conceivably survive for years, but the researchers also found maternal-derived neutrophils. Neutrophils generally live for only a day, says Reed, so they must have been produced by resident stem cells.

Blood brothers

Although it is natural for the blood of mothers and their children to mix during pregnancy, blood transfusions mix the blood of total strangers. Microchimerism has popped up in some transfusion patients as well.

After most blood transfusions, any white blood cells in the donor blood are rapidly cleared by the recipient's immune system, says transfusion medicine specialist Michael Busch of the University of California (UC), San Francisco. But in patients who have received massive transfusions of 10 to 30 units, it is common for donor cells to persist for years. What's more, says Busch, who made the finding with colleagues at BloodSource in Sacramento, California, and the UC Davis Medical Center, the degree of microchimerism is very high. "One [percent] to 3% of all circulating white cells in these patients are of donor origin," says Busch. "It is not one in 100,000 or one in a million like in scleroderma." And the cells that persist all come from just one of the many donors whose blood the patient received. The researchers don't know yet what causes microchimerism in these patients or whether it will lead to autoimmune or other disease.

Indeed, those who study microchimerism in all its forms agree that the discoveries so far have raised far more questions than answers. For example, it is possible that much of the population harbors foreign cells without suffering ill effects. In others the cells might be triggered in some unknown way to do damage. Or the cells in those cases could be innocent bystanders, not culpable in the disease process. "Do we really know that these cells are involved in disease pathogenesis?" Nelson muses. "No." William Reed of Children's Hospital in Oakland, who worked with Busch on the transfusion study, agrees. "This is really just at the observation stage," he says, "with people standing around saying, 'Gee isn't this neat, what does it mean?' "

But that might soon change. The most popular model for how the chimeric cells might cause autoimmune disease is through a reaction akin to GVHD. Until recently, there had been no evidence that chimeric cells taken from their hosts react to the patient's tissues. But in February, a team at the University of Florence, Italy, reported in *Arthritis & Rheumatism* that T cells derived from male offspring in the blood and skin of women with scleroderma could be cultured in the lab and shown to react against the patient's tissue.

CREDIT: A. M. REED, Y. PICNORELL, A. HARWOOD, D. KREDICH, *LANCET* **356**, 2156 (2000)

"This is the most exciting paper that has come out in the last year," says Thomas Jefferson's Artlett. "To me it says these cells are definitively involved." Ann Reed's team at the Mayo Clinic has similar results; her group isolated persistent maternal cells from dermatomyositis patients and showed that they react against the patient's tissues.

Even if foreign cells are shown to respond to the patients' tissues, however, most researchers agree that they can't be carrying out the entire attack themselves; there just aren't enough of them. "Scleroderma looks a lot like graft-versus-host disease," says William Reed, "but the levels of cells you find in those patients is nothing like what you have in graft versus host. Something is missing."

"My premise is that they aren't doing the bulk of the destruction," says Ann Reed; "I think they are the initiator." Once the foreign cells have started an inflammatory reaction, she suggests, the patient's own immune cells are attracted to the scene, where they do much of the damage. Ann Reed is among those who believe that these intruding cells might play an important role in a number of autoimmune diseases. "People said, 'Prove it. Prove they are there, prove that they mean something.' We are slowly doing that. But it takes time."

Lest microchimerism get a particularly nasty reputation, Nelson points out that the majority of people who harbor foreign cells—whether from their children, twin, mother, or blood donor—are healthy. "This is likely to be a broad-based biological phenomenon," she says. "And the best guess, since it is common, is that it may have beneficial roles, it may have neutral roles, and just in selected situations such as a particular lineup of *HLA* genes across generations, it can become bad."

In her talk at the recent meeting, Bianchi of Tufts mentioned two bizarre cases that suggest that microchimeric cells can build tissues as well as attack them. One subject in Bianchi's thyroid study was a 48-year-old mother who had a goiter removed. To her surprise upon examining the removed goiter tissue, Bianchi discovered that one whole section of the woman's thyroid was predominantly male, presumably from her son. Bianchi cites another case, of a woman with hepatitis C who had a liver biopsy. "Part of her liver was entirely male," Bianchi says, "and it was surrounded by female tissue."

Bianchi suggests that, in cases like that of the woman with hepatitis C, circulating fetal stem cells might help repair damaged or diseased tissue. In some cases, rather than cause the disease, she suggests, "maybe the cells are responding to the disease. Wouldn't it be amazing if one of the benefits of being pregnant is that you get, as a reward, a second population of stem cells?" If so, that would be just one more way that mothers and children continue to take care of each other.

–MARCIA BARINAGA

NEWS

Research on Contraception Still in the Doldrums

A billion young people are heading toward their reproductive years, but few new birth control methods are on the horizon

While scientists are beavering away at improving human reproduction, commensurate efforts are lacking on how to curb the process. By 2020, about 1.2 billion people, or 16% of the world's population, will be entering their childbearing years. "We are about to have the biggest proportion of young people the world has ever seen; reproductive health services are about to be inundated by a tidal wave of teenagers," says population expert Felicia Stewart of the University of California, San Francisco (UCSF). "Frankly, I think we're not ready at all." Some 90% of those entering reproductive age will be in the developing world, where there's a particularly pressing need for new forms of fertility control that are cheap, safe, reliable, convenient, reversible, and culturally acceptable.

This should be "a major time for investment" in new forms of contraception, says Stewart, who was formerly in charge of population affairs at the Department of Health and Human Services. But contraception research, which had its heyday in the 1950s and 1960s, hasn't produced a major breakthrough since the introduction of the birth control pill. And there are still only two choices for men: condoms and vasectomy.

Only a handful of companies are engaged in research on new contraceptive methods. One is Schering in Berlin; another is Organon in West Orange, New Jersey, which has just launched a hormone-releasing vaginal ring (NuvaRing), approved in November.

Few options. A Gambian health care worker discusses contraceptive devices, demand for which is expected to grow.

CREDIT: LIBA TAYLOR/CORBIS

But very few others are striving for new breakthroughs. Big pharmaceutical companies left the field in droves in the 1970s, says Carl Djerassi of Stanford University, the father of the birth control pill. Now, he says, "of the 20 largest pharmas in the world, only two have any commitment" to new contraceptives: Wyeth, and Ortho, a branch of Johnson & Johnson. "The only work most are doing is minor modifications" of existing products, he says.

Most companies have been driven away by the same forces at work 2 decades ago: liability worries, tough government regulations in the United States and other countries, and concerns about profitability, a big problem for products where the greatest demand is in poor countries. That leaves governments, international agencies, and private foundations to pick up the tab—with the U.S. government being the number one provider.

Funding has been stagnant for decades. Tellingly, there are no up-to-date figures on global expenditures for contraceptive R&D, and no one has attempted a statistical roundup since the mid-1990s. As a result, there are no more current figures than those in a 1996 report by the Institute of Medicine,* which reported that, in terms of constant dollars, worldwide funding peaked in 1972. And a new report from Johns Hopkins University relates that donors would have to quadruple their efforts to fill the same proportion of contraceptive needs in 2015 as they do now.

Nonetheless, a trickle of products continues to flow into the market, such as the vaginal ring and a new skin patch for women. And a couple of promising new approaches

* *Contraceptive Research and Development: Looking to the Future*, 1996.