Xenotransplants Set to Resume

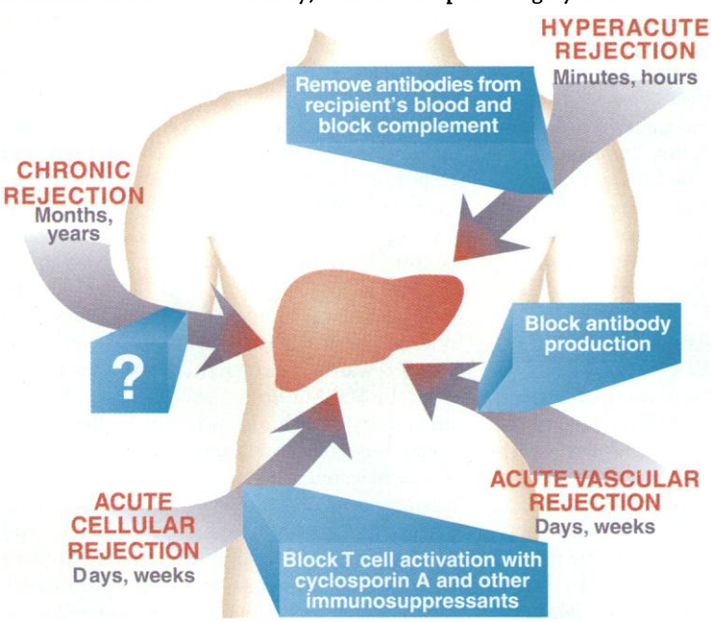
Previous transplants of animal organs into humans have been unsuccessful and controversial. But recent research is encouraging, and the operations may resume in the new year

For a few weeks in 1984 the nation was transfixed by the plight of a 15-day-old baby who had been given a baboon heart because her own had a lethal defect. But the transplant failed, and Baby Fae died at 35 days of age. That spectacular, if unsuccessful, operation split the medical community wide open. One faction saw it as a courageous attempt to offer a critically ill infant a last chance for life. The other saw it as a poorly designed experiment on a human being, destined to fail because so little was known about the powerful immune reactions that reject tissues from another species. Although the split eventually healed, since then, with a few notable exceptions, transplant surgeons have observed a self-imposed moratorium on the use of "xenotransplants," as animal organ transplants are known. The moratorium may soon be lifted, however.

The grim reality that prompted Baby Fae's baboon heart transplant has not changed: "Transplant surgeons are going around metaphorically tearing their hair out, because there are not nearly enough organs to go around," says David White, an immunologist at Cambridge University Medical School in the United Kingdom. This year in the United States alone, up to 3000 patients will die while waiting for hearts, livers, kidneys, or lungs to become available.

Over the past decade, those dire statistics have continued to fan the smoldering interest in animal organ transplants. And in 1995, transplant surgeons—buoyed by a spate of recent successes in research on nonhuman primates—plan to resume baboon organ transplants. But that's not a permanent solution, because baboons are also in short supply. Instead, researchers hope that genetic engineering will soon make it possible to change the pig—an animal whose tissues are far less compatible with the human immune system than the baboon's—into a routine source of organs for xenotransplants.

"The field has become explosively active. I'm amazed at the level of optimism," says Fritz Bach, an immunologist who heads transplant groups at both Harvard Medical School and the Vienna International Research Cooperation Center in Austria. Mark Hardy, head of transplant surgery at Columbia University College of Physicians and Surgeons in New York City and president of the American Society of Transplant Surgeons, offers an explanation for the upbeat new mood: "The field is beginning to mature. The reactions of xenotransplant rejection are being analyzed, finally," he says.



Slipping the defenses. Getting successful xenotransplants in humans will require overcoming four types of rejection barriers.

That rigorous new approach has already won converts. The National Academy of Sciences' Institute of Medicine (IOM) is setting up a committee to examine xenotransplantation (see box on p. 1149). And, for the first time in its 90-year history, xenotransplantation seems promising enough for the pharmaceutical industry to invest heavily. "You can certainly still find purist immunologists who say it can't work, but [that] community is getting smaller," says François Meyer, a Sandoz vice president who oversees the company's investments in xenotransplant research. Nevertheless, as Bach warns, there are still "an awful lot of unknowns" to overcome.

Those who favor resuming baboon organ transplants argue that the few stabs at primate xenotransplantation have been far more successful than is generally supposed. As early as 1963, Keith Reemtsma, then at Tulane University Medical Center in New Orleans, transplanted chimpanzee kidneys into six patients with advanced kidney failure. That was long before the introduction, in the 1980s, of cyclosporin A, a drug that helped change human organ transplants from a high-risk activity to a mainstream therapy. Even without that drug, Reemtsma, now also at Columbia, found that chimp kidneys were no more likely to be rejected than were human kidneys. One patient lived out of the hospital for 9 months before she died.

Baboons as organ donors

Transplant surgeons subsequently switched to baboons because the chimpanzee is an endangered species. But even with baboon organs, the intensity of the rejection "is probably far less than most scientists would expect," says Steven Gundry, who now heads the cardiothoracic surgery division at Loma Linda University Medical Center in California, where Baby Fae was treated. Indeed, Baby Fae's heart functioned for 20 days until her death, which was due not to the xenotransplant per se, he says, but to the fact that she belonged to blood group O, while the baboon donor had type AB blood, a mismatch that usually precludes a transplant. And in 1992, a 35-year-old man who received a baboon liver transplant at the University of Pittsburgh School of Medicine survived for 2.5 months, dying not from rejection, but from an infection, the result of the drugs used to suppress his immune system.

But the most encouraging evidence that cross-species transplants between primates can be tolerated comes from animal studies. Robert Michler, chief of cardiac transplant at the Columbia Presbyterian Medical Center in New York City, and his colleagues have transplanted hearts from cynomolgus monkeys into six baboons and achieved an average survival time of 6 months. In Michler's view, that success isn't sufficient to justify attempting to use baboon hearts as permanent replacements for human ones, so he's working on a technique for using the baboon heart as a temporary "bridge," although he hopes that what he learns from

Animal Organ Transplants Raise Ethical Issues

The imminent prospect of "xenografting," or transplanting animal organs into human patients, raises not only a host of scientific questions (see main text), but a plethora of social and ethical issues as well. Among them: How to protect the rights of the first "pioneer" patients? How to prevent the introduction of dangerous animal pathogens into the human population? And will the public find the idea of transplanting animal organs into humans acceptable? The National Academy of Sciences' Institute of Medicine (IOM) is concerned enough about these issues to convene a committee that will report next fall.

One of the hottest questions to face the IOM committee—and the institutional review boards that have the task of deciding whether to approve clinical trials of xenografting—will be how to select the first patients for procedures that, at least initially, are unlikely to benefit the patient. One choice is the "patient-pioneer," individuals who have exhausted all other therapeutic options and opt for experimental surgery knowing the chances of success are slim. The use of "human guinea pigs" is morally defensible, says Arthur Caplan, director of the center for bioethics at the University of Pennsylvania, "as long as the patients understand how desperately unlikely it is that they will benefit."

David Cooper, director of research at the Oklahoma Transplantation Institute in Oklahoma City, sees a better way. He suggests using the first xenografts as "bridges" that will tide patients over until human organs become available for transplantation. "You have to link your research to some reasonable prospect of benefit [to the patient]. If you can say that we reasonably expect to get a few weeks out of this [organ], so we will put it in and look for a human organ to replace it as soon as we can, that would be a way to gain experience."

Another concern is that every attempt at xenografting be "done in such a way that the field can learn from it," a principle that hasn't always been followed in the past, says Jeffrey Platt, immunologist and professor of experimental surgery at Duke University Medical School. "There has to be a rigorous analysis of the pathological outcome and the immunological reactions," he says, and "it is critical that it be published [so] that the field can benefit."

And whatever organs and patients are finally selected for clinical trials, the advent of clinical trials of xenografting will no doubt raise the specter of transmission of animal pathogens into humans. To illustrate the possible impact, infectious-disease experts point out that the AIDS virus probably originated in a nonhuman primate. To help protect against such risks, "it's important to do [xenografts] cautiously," and to screen each donor animal for potentially disease-causing pathogens, says the University of Pittsburgh's transplant infectious disease expert Marian Michaels.

But that's easier said than done, as it's impossible to screen for animal pathogens that haven't even been discovered yet. The AIDS virus, for example, wasn't identified until many human beings began to show serious symptoms, which probably appeared years after the initial infections. As a result, Michaels says, patients will have to be monitored for signs of infection by exotic viruses as long as they live. And if xenografts become routine, it will be necessary to create germ-free colonies of donor animals.

Here, pigs have the edge over primates. Because pigs mature early sexually, have large litters, and grow rapidly, they are easier and cheaper to breed in germ-free environments than are primates. And fewer people are expected to find it objectionable to farm pigs for organs.

But even if people aren't overly concerned about farming pigs, medical ethicists worry that they will find the use of pig or baboon organs too "unnatural" to be acceptable for human transplantation. Such aversion to procedures that medical ethicists believe are morally defensible is known in bioethics circles as the public's "gag factor." Caplan argues, however, that "naturalness" is not intrinsic to a medical procedure but a function of familiarity. A century ago, he says, many people found the idea of anesthesia and surgery "unnatural." For xenografting, however, the day when it seems natural may be some way off, to judge by the experience of Fritz Bach, head of xenotransplant research programs at both Harvard Medical School and the Vienna International Research Cooperation Center in Austria. He made time in a busy schedule to stop over at the Munich airport to explain xenografting to a German television reporter, to no avail. "So ugly, so upsetting was [the concept] to her, she actually passed out right in front of my eyes," he says.

–R. N.

these studies will eventually make permanent baboon heart transplants possible.

In the past year, Michler and his colleagues transplanted hearts from cynomolgus monkeys into five baboons and 2 weeks later removed the monkey hearts and replaced them with other baboon hearts. Besides ensuring that there was a good tissue and blood match between the donors and recipients for all the transplants, the researchers adopted a two-pronged strategy to prevent rejection of the monkey hearts. To inhibit acute cellular rejection, caused by the immune system's T cells (see diagram on p. 1148), they used cyclosporin A. And to prevent acute vascular rejection, triggered by the slow buildup of antibodies to the foreign tissue, they removed the baboons' spleens, the main source of the antibodies, and treated them with cyclophosphamide, a drug that inhibits the antibody-producing B cells.

None of the monkey hearts were rejected; nor did they sensitize the baboons to the second transplant. The baboon hearts "looked perfectly normal when we removed them 2 months later," says Michler, who is sufficiently encouraged by the results that he has applied to his Institutional Review Board (IRB) for approval to start using baboon hearts as last-resort bridges for patients who become critically ill while on the human heart waiting list. "We expect approval by the end of the year, and we hope to start the program on January 1," he says.

Other transplant centers, meanwhile, hope to go straight to clinical trials to test the permanent use of baboon hearts in humans. In the past 3 years, the Loma Linda team has transplanted 17 rhesus monkey hearts into baboons. By trying various combinations of cyclosporin A or another T cell inhibitor, FK506, and methotrexate, a drug that also inhibits B cells, the team has hit on a regimen in which the number of rejections and infections "are the same as in humans undergoing regular heart transplants," says Gundry. It is still too early to say how long the baboons will survive, because some received their hearts only 6 months ago. But if the baboons thrive for another 6 months, Gundry's team will apply to Loma Linda's IRB to start clinical trials to test baboon hearts as permanent transplants in humans. Meanwhile, there are also plans to test baboon bone marrow as a therapy for AIDS (see box on p. 1150).

But even if transplants of hearts and other baboon organs into humans succeed, they "won't solve the shortage problem," warns Jeffrey Platt, immunologist and professor of experimental surgery at Duke University Medical School. Each year in the United States, up to 43,000 patients with heart fail-

Immune Reconstitution for AIDS

Is it possible to cure late-stage AIDS by reconstructing the patient's ravaged immune system with that of a baboon? In theory, there's reason to think this bizarre-sounding proposition could work, because baboons are resistant to infection with the main AIDS virus, HIV-1. And the theory will soon be tested, if a proposal before the ethics review board at the University of California, San Francisco (UCSF), gets the go-ahead.

The proposal to transplant baboon bone marrow into four late-stage AIDS patients is the brainchild of transplant surgeon and immunologist Suzanne Ildstad of the University of Pittsburgh, who's teamed up with UCSF AIDS researcher Paul Volberding to spearhead the operation.

Several groups, including Ildstad's, have already shown in a string of experiments in rodents and primates that it is possible to graft the immune cells of one species onto the immune system of another without the long-term use of immunosuppressive drugs. Now, the Ildstad team has created a baboon whose immune cells are 15% human. The procedure used concentrated immature marrow cells and required only low levels of radiation to prepare the recipient's bone marrow for the graft and 2 to 5 days of immunosuppressive drugs. The baboon received the human marrow transplant only 3 months ago, so it's not yet clear whether the grafted immune cells are functional. But, says Ildstad, they look normal, and the baboon tolerated the procedure without apparent ill effects.

Moreover, Volberding says, the protocol for the clinical trial has been designed to minimize risk to the patients who, because they are infected with HIV, are particularly sensitive to the toxic effects of irradiation and immunosuppressive drugs. The first patient will receive extremely low doses, and only if these are tolerated will the next patient undergo the procedure, using slightly higher doses if the first patient's graft doesn't take.

Even with this cautious approach, some AIDS researchers argue that human trials are premature. "No one wants to say 'no' to something that might potentially be helpful," says HIV virologist Mark Feinberg of the Gladstone Institute in San Francisco. But, he argues, more animal experiments are needed before going into human trials. "We're [not] doing anyone a favor by not taking on the hard issues and trying to hit the grand slam," he says. Specifically, Feinberg and other critics of the baboon marrow transplants point to several questions they would like to see answered before human trials begin. Is the damaged immune system of an AIDS patient capable of "educating" the baboon immune stem cells so that they tolerate human tissue, or will the transplanted immune system attack the tissues of the host? Will the baboon immune cells be able to mount effective attacks on agents that infect AIDS patients? Will the irradiation and immunosuppression needed for engraftment make the patients even sicker? And if the graft doesn't take, will the immunocompromised patient be left fighting off a variety of baboon viruses?

Those questions could be partially addressed in monkeys infected with the AIDS virus relative SIV. But advocates of the human trials note that such experiments are far from perfect indicators of what would happen in AIDS patients. What's more, they would take 2 or 3 years, during which time more AIDS patients would die. And that helplessness is what impels Volberding. He concedes that the odds of the baboon marrow transplants benefiting AIDS patients are low, but he says "there is a chance that it is going to be helpful, as opposed to ... our current drugs that really don't work."

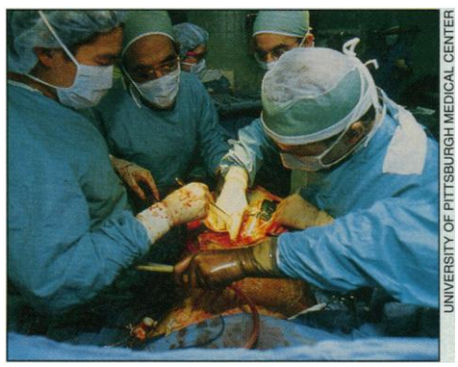
–R.N.

ure are denied potentially life-saving transplants because of the organ shortage. In contrast, the world's largest captive baboon colony (at the Southwest Foundation for Biomedical Research in San Antonio) houses only 2700 baboons. So without resorting to factory farming—a notion repugnant to researchers, surgeons, and the public alike—baboons cannot meet even the need for hearts.

Battle of the immune systems

To fill the gap between supply and demand, researchers are pinning their hopes on the pig. "The focus is on the pig," says Hardy, because the pig is in plentiful supply; "it comes in all sizes; it can be raised in a [specific pathogen-] free environment; and it has a similar physiology to man in terms of its heart, liver, and pancreatic islets." Those factors all make the pig a likely candidate, but there's a major downside to using pig organs: In the human body, pig organs trigger an explosive immune reaction called hyperacute rejection.

This type of rejection has two main components. One is a type of antibody found in the blood of every human that rushes in the moment a pig organ is connected to the blood supply of a primate and anchors itself to the surface of the endothelial cells lining the blood vessels of the xenotransplant. Once these "xenoreactive" antibodies have attached, they lure into the organ one of the most primitive battalions of the immune system—the complement proteins. The complement proteins stab holes in the membranes of the endothelial cells and in unison with the antibodies disrupt the endothelium so that the blood clots in the vessels. Under the combined onslaught, the transplanted pig organ becomes black, swollen, and clogged with congealed blood. This is hyperacute rejection; it will destroy the organ within minutes.



Mix and match. University of Pittsburgh Medical Center surgeons perform a baboon-to-human liver transplant.

One tactic for blocking the hyperacute rejection of pig organs is to sop up the xenoreactive antibodies by passing the recipient's blood through a pig kidney that is not intended for transplant or a filter that contains the appropriate antigen. This approach has worked in animal experiments, says Platt, "and even when the antibodies return to the circulation [a few days later], they [don't] necessarily destroy the organ," which adapts by some as-yet-mysterious process to its new environment.

But even in the absence of xenoreactive antibodies, complement proteins still lead to organ rejection, although the effect takes days rather than minutes. Consequently, competing teams are using the tools of molecular biology, trying to bestow on pigs some of the same defenses that protect primate organs from human complement proteins. Among these defenses are a protective armor of proteins, including decay accelerating factor (DAF), membrane cofactor protein (MCP), and CD59, which are located on primate cell surfaces.

Some believe that pig organs could be protected if they are modified to produce these proteins. "Our view is that you should

change the pig—not the patient," says Cambridge's White, co-founder of the Sandoz-sponsored xenotransplant company Imutran. White and his co-workers have genetically engineered pigs so that their cells carry human DAF on their surfaces and found that the transgenic pig's heart continues beating for up to 4 hours while being perfused with human blood—a dramatic improvement over the unmodified pig heart, which dies within minutes.

Platt's team, working with researchers at Nextran, a Princeton, New Jersey, biotechnology company, whose major stockholder is Baxter Healthcare Corp., has also created several families of transgenic pigs that express various combinations of DAF, MCP, and CD59. One of the earlier pig lines expressed the proteins only at low levels. Nonetheless, when researchers transplanted hearts from these pigs into three baboons (whose complement response is similar to that of humans), the hearts continued beating for between 4 and 30 hours.

Subsequently Nextran and the Platt team have honed their transgenic approach, says Nextran's vice president of research, John Logan, and next year they will test a new strain of pig that permanently expresses complement inhibitors in the hopes that transplanted hearts from these animals will survive much longer in primates. Despite the hope they offer, genetically engineered pigs alone are "not going to make xenotransplantation suddenly possible," says Logan. "This is going to be just one in a series of steps."

And, most researchers agree, 1995 will be the pivotal year for deciding how rapidly the next steps can taken. They hope that by using the transgenic pigs they will be able to see what can be achieved by blocking the hyperacute rejection. Among the questions to be answered are: Will pig organs function in a human body? Will genetically engineered pig organs be subject to an acute vascular and cellular rejection that will prove more difficult to handle than those triggered by human organs? And will they be subject to the little-understood chronic rejection that, even in the face of apparently adequate immunosuppression, strikes human organs years after transplantation?

Even if those key scientific questions can be answered, there's one more potential barrier to success. "The fear is," says Platt, that some transplant surgeons "will be cavalier in their approach ... and cast a shadow over the whole field"—just as the Baby Fae episode did. That's where the IOM report could help, as its new committee plans to highlight the ethical issues associated with xenotransplantation. IOM's Valerie Setlow argues that these issues should be considered now, because, she says, when it comes to xenografts, the main question is "not if, but when."

–Rachel Nowak

INDUSTRY COLLABORATIONS

Networking Gene Therapy

If you think about the most successful technology-based companies of the 1970s and '80s, the name Microsoft inevitably comes to mind. By meshing the disparate technologies of personal computing, Bill Gates made that company a runaway success. Now the U.S.–French pharmaceutical company Rhône-Poulenc Rorer (RPR) says it will try to emulate this approach for one of the key emerging technologies of the 1990s: cellular and genetic therapies. This week RPR announced that it has set up a network of 14 partnerships with labs and companies in these fields; the company will spend at least $100 million per year over the next several years supporting their research.

RPR's announcement follows similar initiatives by other major pharmaceutical companies—including SmithKline Beecham, Glaxo, Roche, and Eli Lilly—to join forces with academia and start-up biotechnology companies in tackling gene-based research. But the new network is unique in bringing together so many groups with a focus on gene therapy, which remains an unknown quantity commercially.

Various groups around the world are attempting trials of gene therapies for cystic fibrosis, but RPR is the first company to launch such an aggressive attack on more common diseases—its targets are cancer, atherosclerosis, and central nervous system disorders such as Alzheimer's disease. "All of [RPR's] incremental research and development [spending] has gone on gene therapy [and related biotechnologies]," says pharmaceuticals analyst Ian Smith of Lehman Brothers stockbrokerage in London. "They've basically taken a bet with themselves that this is the way ... to carve out a leading-edge position in gene therapy. It takes courage to do what they're doing."

RPR has risked a big stake on that bet: By the end of the year the company will have plowed $300 million into setting up the network, which will be focused around a new division of the company, RPR Gencell, with headquarters in Collegeville, Pennsylvania, and a staff of 150. The range of problems to be tackled is unprecedented: Partners in the network are studying the roots of diseases, characterizing the gene or genes involved, and going back to treat the diseases with gene-based therapies.

The company has also opted to form a loose federation of partnerships, rather than taking over companies or labs. So far, all the partners are based in France or the United States, homes of RPR's two headquarters. The researchers receive an injection of cash and placement of RPR staff or funds for staff and technicians, followed by later milestone payments depending on performance; in return they sign over to RPR equity, rights on specific projects, and first right of refusal or negotiation over products arising from the joint effort. "We want to split the responsibility, let academic researchers do what they do best—get proof of the principles—then make the connections [with industrial expertise]," says Jean-Bernard Le Pecq of RPR Gencell.

Such a flexible arrangement is more attractive than a series of mergers, argues David Nance, president of Introgen Therapeutics, a network partner, because "sometimes a big company can take over a small biotech company and all the things that were exciting and desirable about the small company seem to dissolve." The 14 partnerships agreed to so far will not be the whole story, says Le Pecq, because RPR will seek new collaborations "any time some team in the world find a new principle."

Scientists involved are enthusiastic about the network. The work is "collaborative ... it's not limited to funding, but it's an exchange of information," says virologist Jean-Michel Heard of the Pasteur Institute in Paris. RPR funds two scientists from Heard's team of 12 working on ways to replace the protein that is missing in lysosomal storage disease. This condition is the result of a defect in one gene, so reinserting the functional gene would result in re-expression of the protein. But it would be impossible to deliver the DNA to the relevant cells in all the affected tissues—liver, spleen, bone, and joints. Instead, Heard's team aims to insert the gene into other cell types, such as fibroblasts or muscle cells, so that they express the missing enzyme and secrete it into the serum, from where diseased cells can absorb it. RPR is interested in how this technology could be used to deliver proteins that alter the metabolism of cholesterol into the serum constantly, helping to clear lipoproteins from the blood.

It isn't only those involved in the network who tout its benefits. Outsiders agree that loose federations may be the way to go in large collaborations involving academic labs and companies. Dinko Valerio, the head of IntroGene, a small biotech company in Rijswijk, the Netherlands, working on gene transfer in precursor blood cells, believes "the organization of such a large network could be difficult ... [but considering] all the different technologies and know-how that have to be combined in order to get gene therapy to work ... it sounds like it's the right thing to do."

–Claire O'Brien

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