That’s Some Pig

BY ALLAN COUKELL

Some time in the 1600s, a sword-wielding Tartar cracked open the head of a Russian nobleman named Butterlijn. Desperate for something to repair the wound, an enterprising surgeon borrowed a suitably sized piece of bone from the skull of a dead dog. Butterlijn recovered. But when word of the remarkable achievement reached the archbishop of the Orthodox Church, he threatened the nobleman with excommunication. Butterlijn fled.

This story, even if apocryphal, marks an early episode in the long, strange and mostly unsuccessful history of xenotransplantation—the practice of introducing animal tissues and organs into humans. Yet after hundreds of years of failure, modern medicine may finally be close to solving the transplant problem. The need has never been greater.

At any given moment, some 89,000 patients in U.S. transplant centers require an organ, according to the United Network for Organ Sharing; many die waiting. And the list grows daily. So scientists dream of organ farms and an endless supply of healthy hearts, kidneys, livers and lungs. It is a vision David Sachs has been pursuing for more than 30 years. But bridging the species barrier has been harder than he expected. As a young immunologist at the National Institutes of Health in the 1970s, Sachs was aware of efforts to use chimpanzee kidneys to save the lives of patients with advanced renal failure. But to Sachs, the ideal cross-species donors were pigs, not primates.

Despite the close genetic similarity between chimpanzees and humans, pigs offer the advantages of being plentiful, fast-breeding and easy to keep. Their hearts, kidneys, lungs and livers are about the same size as human organs. And while there would be ethical objections to using humans’ close primate relatives as organ donors, it seemed to Sachs that few would mind using pigs, millions of which are butchered annually for bacon.

Scientifically, though, there are issues. Over millions of years, human beings have evolved a system of immunological defenses exquisitely adapted to repel foreign invaders. The same system that identifies and destroys bacteria and viruses also attacks any other living matter the body recognizes as “nonself.” Pigs are certainly nonself.

When transplant recipients reject human organs, it is normally because the body—specifically a type of white blood cell, called a T-cell—has recognized the foreign tissue. To minimize the difference between donor and recipient, doctors check for blood-group compatibility, as they do for transfusions. They also consider tissue type, using as markers a set of molecules called MHC proteins. The closer the MHC match, the less likely it is that the transplant will be rejected. But once T-cells recognize a foreign MHC protein, they set off a cascade of responses that, unless it’s suppressed by drugs, results in the failure of the organ.

Unfortunately, pig organs are rejected much more swiftly than human ones. A pig kidney transplanted into a primate will almost immediately begin to darken and turn black. “You look inside and you see bleeding, hemorrhage, thromboses everywhere,” says Sachs, now director of the Transplantation Biology Research Center at the Massachusetts General Hospital. “Often the organ is dead before the surgeon completes the procedure.”
The reason for this violent reaction remained a mystery for many years. Scientists knew it was caused not by T-cells but by circulating antibodies. (Antirejection drugs, which mostly reduce T-cell activity, are useless against this kind of rejection.) But it was only in the early 1990s that the culprit behind the response—a type of sugar called alpha-1,3-galactose, commonly known as Gal—was identified.

Most mammals, including pigs, “express,” or manifest, Gal; it is found on the surface of nearly every cell and in the lining of blood vessels. But old-world primates, including humans, do not express this sugar and thus react strongly to it. This discovery launched a race to produce Gal-free pigs, whose organs might not be summarily rejected.

It was around this time, in 1993, that Jennifer Searl got sick. A petite 12-year-old with light brown hair and green eyes, Searl was a scrappy soccer player who, her mother had noticed, was looking a little pale. She was on her way to summer camp when the results of a blood test explained why: Her kidneys had failed.

A transplant from Searl’s father followed, but problems started almost immediately. Her system weakened by antirejection drugs, she was soon dealing with a nasty viral infection. Her body also began to reject the kidney, and the side effects of the drugs were devastating. Painful, wartlike growths covered her right foot. Ten times they were removed and 10 times they grew back. She could hardly walk. Her choice seemed to be life on dialysis or another transplant with still more drugs.

Though Searl’s first transplant failed, it wouldn’t even have been tried without two significant medical advances: tissue matching and the development of antirejection drugs. But by then Sachs and his colleagues were pursuing a third approach to the transplant problem.

In the late 1940s, scientists showed that the ability to distinguish “self” from “nonself” develops around the time of birth. A few years later, the British zoologist Peter Medawar discovered that if he injected a mouse fetus or newborn with cells from an adult mouse, he could later transplant skin from the second mouse to the first without causing rejection—an observation that helped him earn a Nobel Prize. This process, called “tolerance,” was the subject of a lecture that had first drawn Sachs into transplantation research.

At the National Institutes of Health, and later in Boston, Sachs spent years trying to induce tolerance in adult animals. Working first with mice and later with pigs and monkeys, he learned how to temporarily destroy an animal’s immune system, then “rescue” it with bone marrow from another of the same species. When the marrow recovered, it contained a mixture of T-cells from both individuals. Sachs found that the first animal would then accept an organ from the bone marrow donor, without rejection and without drugs.

For Searl, life only got worse as her body continued to reject the transplanted kidney. The drugs caused not only warts but also cataracts and bone wasting, and her teenage face became grossly swollen. Her mother, although not an MHC match, was a potential donor for a second kidney transplant, but Searl doubted that she could tolerate more of the same.

The combined bone marrow and kidney transplants Sachs had developed in animals had been successfully carried out with a handful of people, all of whom had multiple myeloma, a cancer of the bone marrow that often causes renal failure. The procedure hadn’t been tried on someone without cancer. Searl was to be the first.

In September 2002 she was admitted to the Massachusetts General Hospital. She received radiation to her chest and chemotherapy that partially destroyed her bone marrow. Then a drug called MEDI-507 depleted her body of T-cells. A week later, she was wheeled to the operating room, where some of her mother’s bone marrow cells were infused into her veins through an intravenous tube, and she then received her mother’s kidney.

Compared with her previous transplant, the short-term risks of infection and death would be expected to be higher with this...
Over millions of years, porcine endogenous retroviruses (PERVs) have taken up residence in the DNA of pigs. These dormant viruses don’t bother the pig. But what if, transplanted with an organ into a different species, a PERV were reactivated and became pathogenic? That’s the worst-case scenario for xenotransplantation from pig to human—that it could give rise to a new cross-species disease.

Any transplant, including human to human, carries some risk of infection, and several pig diseases can also infect people—swine flu, for example. Yet while most such cross-species transmissions can be controlled or eliminated, there has been concern that PERVs could make the trip from pig to person.

That risk caused the U.S. Food and Drug Administration to place all xenotransplantation trials on hold in 1997; various European governments took even stronger action. That year, the British virologist Robin Weiss showed that, under some conditions, PERVs can infect human cells grown in the lab. This risk might be increased in someone whose immune system has been suppressed by antirejection drugs. Weiss also argued that Gal-free pig organs, by circumventing another layer of defenses, might be more efficient conduits for viruses.

Tests of patients who have been given pig brain, liver or insulin-producing cells have so far shown no evidence of PERV infection. Pig-to-baboon transplants have also come up clean. And xenotransplantation researchers are now using various genetic techniques to disable the PERV DNA. For anyone considering a lifesaving transplant, the risk seems minuscule.

But policymakers have to think not just of individuals but of the entire population. The trials placed on hold in light of Weiss’s research findings were allowed to continue once they satisfied revised FDA requirements. Weiss, who was “very impressed” with the FDA response, nevertheless worries now that the agency may have gone too far. “If regulators are too precautionary,” Weiss warns, “they are going to stifle the whole technology.” Still, Weiss doesn’t regret having raised the alarm. “I really did think people needed to wake up to the danger.”
procedure. But this time, everything worked. Ninety days after she went into the hospital, Searl was off all antirejection drugs.

It is too soon to say whether tolerance will lead to long-term survival of transplanted organs in humans, but the signs are good. One of the first myeloma patients still has a functioning kidney six years after transplantation. What’s more, one of Sachs’s early baboons has gone 10. (“We don’t know what to do with him,” Sachs says. “We’re paying per diems, and he’s just normal!”)

Jennifer Searl Duran, three years after her second transplant, is newly married and training to run her first marathon.

As useful as the tolerance approach may be for human-to-human transplants, Sachs believes its real potential is in xenotransplantation. The birth of Dolly the sheep, the first mammal to be cloned from an adult cell, ushered in the era of cloning that made it possible to produce pigs without the Gal gene. Pigs are somewhat harder to clone than sheep, but in 2002 a group of scientists led by Randall Prather at the University of Missouri, Columbia, reported using pigs that Sachs had bred to produce offspring which had one of the two Gal genes “knocked out.” The scientists grew cells in culture in the laboratory, replacing the Gal gene with a similar but nonfunctional sequence of DNA. Then they used the nucleus of one of those cells to create the first Gal “knockout” clone. Using similar methods but different pigs, a second research group achieved the same milestone not long after. Soon scientists were able to produce pigs in which both Gal genes were knocked out, allowing them to breed Gal-free pigs.

By bringing together the two strands of research—the Gal-free pigs and the tolerance approach to transplants—Sachs hoped to eliminate both major barriers to xenotransplantation. He began making preparations for a series of pig-to-baboon transplants using, instead of a bone marrow transplant, a second method of inducing tolerance that entails transplanting part of the thymus, the gland that produces T-cells.

In earlier experiments, survival after a pig-to-baboon kidney transplant had never gone beyond 30 days, even when anti-Gal antibodies had been filtered out of the blood. In contrast, baboons that received a Gal-free pig kidney, combined with the thymus procedure, lived as long as 83 days. The longest-lived animal in this 2005 study died of a heart attack with its kidney apparently still in good shape. Sachs was thrilled. “I’ve never seen a pig-into-baboon kidney transplant—nobody has!—on day 83 that looked that normal.”

Others, though, are skeptical. Jeffrey Platt, head of the Transplantation Biology Program at the Mayo Clinic in Rochester, Minn., and another long-time xenotransplantation researcher, thinks the Gal-free pigs will teach important fundamental lessons, but questions whether they will really provide better transplants. “I have yet to see evidence that they do,” he says. “Other researchers using pigs that do express Gal have gotten results comparable to the Boston group’s.” He likens Gal to the antigens responsible for blood type

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incompatibility, which Platt says have become usually manageable transplant problems.

David Cooper, who used to work with Sachs and is now at the Thomas E. Starzl Transplantation Institute at the University of Pittsburgh Medical Center, believes the Gal-free pigs are a major advance. But Cooper has likened the transplantation problem to peeling an onion—remove one layer and another presents itself. Since overcoming the hyperacute rejection barrier, the scientists have been seeing a new phenomenon in pig organs transplanted into baboons. Instead of the sudden blackening and shriveling, numerous tiny blood clots begin to appear after about a month.

Cooper and Sachs differ over the clots. Cooper believes they might result from differences between the pig and primate coagulation systems. His group has again turned to genetic engineering to put human anticoagulant genes into their experimental pigs. Right now, Cooper says, “we are all waiting for the next version of pig.”

Sachs isn’t waiting. He saw many fewer clots in the baboons that received Gal-free kidneys plus the tolerance procedure than in those that received only standard immunosuppression, leading him to believe that his tolerance approach may render the problem irrelevant.

The imminent arrival of xenotransplantation has been predicted for years, but the science isn’t quite there yet. The companies that poured money into the field in the 1990s have now largely withdrawn, turning the intellectual property over to the universities.

Questions linger: What about the chronic rejection that claims about half of human tissue grafts within a decade? With xeno, will that be less of a problem, or more? Will there prove to be another, unanticipated layer of rejection? What about the small but potentially catastrophic risk of new diseases (see “Crossing the Line,” page 41)? And if rejection can be overcome, will pig organs function well enough to give people an acceptable quality of life?

Sachs is confident. “I think we’re very close,” he says, sitting in his ninth-floor office with its view of Boston Harbor. Then he leans back and smiles, a touch ruefully. “I just don’t know for sure. I thought we would have had all of this done 10 years ago.”