
THOMAS

THOMAS STARZL SAYS TOLERANCE IS ON THE OTHER SIDE OF REJECTION

BY CHUCK STARESINIC

BREAK ON THROUGH

CONTINUED FROM OUR MAY ISSUE

In 1986, Thomas Starzl flew to Japan for a chance at a few drops of an experimental drug known by the unwieldy designation of FR 900506. The response to his inquiry was, essentially, "Maybe." After Starzl's formal request, a Japanese pharmaceutical executive immediately jetted off to London to talk things over with officials from a British company with which his firm collaborated. Starzl, then a Pitt professor of surgery, waited in Japan for a definitive answer.

He was 60 years old. He had a reputation for boundless intellectual and physical stamina, not to mention unparalleled skill and drive as a surgeon. He was the man who had made liver transplantation a reality when many had insisted it could not be done. But now he felt chronically fatigued. He was becoming careful instead of bold, he would later reflect in his 1992 memoir, *The Puzzle People*. And he privately wondered whether this drug was better left to someone else to champion or reject.

Researchers at the Thomas E. Starzl Transplantation Institute believe that decades of laboratory research and clinical experience will yield a systematic way to help transplant patients tolerate their new organs with little or no medication. One avenue of investigation reaches into the depths of the sea for answers. Oddly enough, jellyfish that live on the backs of hermit crabs have offered clues.

ILLUSTRATION | JACOB THOMAS AT DECO ZONE

In Pittsburgh, transplant patients carried on the all-consuming struggle to live. Drained of color and vigor, jaundiced, incoherent, and perhaps even comatose, they waited for organs without which they would die. Families wrestled with the knowledge that their prayers could only be fulfilled through someone else's tragedy. The lucky ones weathered the aftershocks of transplantation: Their tissues were inflamed as a result of incisions and the stitching together of foreign blood vessels and ducts. More ominously, their white blood cells—roving protectors of the status quo that are born in the bone marrow—diligently attempted to seek out, identify, and destroy any cell from the donor organ so carefully stitched into place.

It was on behalf of these patients, and all those to come after, that Starzl, now a Distinguished Service Professor of Surgery, waited in Japan for one-hundredth of an ounce of FR 900506. Starzl's patients in Pittsburgh were being treated with cyclosporine then, a drug that Starzl had begun experimenting with in 1979. When he first demonstrated to the medical community that a careful blend of cyclosporine and steroids could control the assault of the immune system upon a donor organ, the field of transplantation took an enormous leap forward. Survival rates surged upward. Unfortunately, cyclosporine was somewhat toxic. It could lead to serious kidney damage or outright failure. Some patients in early trials without steroids had developed white blood cell cancers. Starzl avoided such catastrophes through the addition of steroids and by administering the lowest dosage possible, but some patients rejected their new organs despite the help of cyclosporine.

Standing in the lobby of the Fukuoka Hotel one week after his arrival in Japan, Starzl got his answer. At the end of a slow, almost ceremonial discussion translated by a Japanese surgeon, Starzl was entrusted with a vial of liquid FR 900506. The contents would not have filled a thimble. He rushed back to Pittsburgh with it and with the promise of more.

Like cyclosporine, FR 900506 (which now goes by the names FK 506 and tacrolimus) suppressed a subset of white blood cells that helped the immune system target the new organ for destruction. Researchers at other institutions cautioned Starzl that it was prohibitively toxic—a probable dead end, they told him. But Starzl had patients who were running out of options, and he believed that, like cyclosporine, this drug could work if carefully combined with steroids.

Eventually, tacrolimus was approved for patients who had rejected multiple organs—they were facing either another risky transplant or death. Starzl's first was a woman rejecting her third liver in eight months. Then came a man rejecting his fifth liver in four years. Tacrolimus stopped rejection in both of them. After two years, seven of the first 10 people who'd switched from cyclosporine stopped and reversed a deadly immune response. Patients with failing transplants came to Pittsburgh from around the world.

Tacrolimus is used widely to this day, yet it's long been clear that suppressing the immune system with highly toxic drugs is an imperfect way of making transplants stick. Patients who are saved by new livers or kidneys are forced to endure side effects like debilitating pain, nausea, tremors, excessive hair growth, high blood pressure, diabetes, and tumors. You could survive a liver transplant at 35, but die at 50 from the side effects of your medication.

When Starzl came to Pittsburgh in 1981, his goal had been to prove that organ transplants were possible on a large scale, especially bloody and technically challenging liver transplants. He had accomplished that in Pittsburgh. Now, there was a new peak to bag: tolerance—a state in which an organ would be accepted without immunosuppression.

Tolerance was not complete fantasy.

As early as the 1960s, Starzl had taken patients off immunosuppression on a hunch. A few showed no ill effects and remained drug-free. Those who showed signs of rejection went back on their meds. Yet the science of immunology had no clear explanation for why one patient could achieve tolerance and another would fail to. To add to the mystery, it would later become clear that patients who'd had transplants in the 1960s were more likely to achieve tolerance than those transplanted later, say, in the 1990s. While surgeons had gotten better at doing transplants and had found better drugs to stop rejection, their patients seemed less likely to achieve tolerance.

Starzl walked out of the operating room one day in 1990 and decided he wasn't going back. He was done salvaging healthy organs from those unfortunate enough to no longer need them. Done standing all night sewing livers into those who would die without them. He found it a great relief.

With more time on his hands, Starzl decided to scratch a mental itch he'd had for years concerning his most successful patients. In 1992, he invited 30 of the world's longest-surviving transplant patients to Pittsburgh. Among them were a man who had lived 29 years with someone else's kidney and a woman who had lived 23 years with a transplanted liver. The 30 were on various levels of maintenance immunosuppression, and at least one was drug free.

According to the prevalent immunological theories of the day, their donor organs were islands amid hostile seas. To keep the immune system from swelling into a tidal wave, doctors calmed the seas with drugs. Cells from a donor organ that dared to set foot off the island invited destruction. Starzl suspected there was more to the story than that. He asked his patients to provide blood and tissue samples from several parts of their bodies, even biopsies of various organs. He then enlisted the help of Pitt experts in, among other techniques, the sort of DNA fingerprinting that allows criminal prosecutors to identify an individual from the DNA in a tiny trace of blood or a hair.

Whom did Starzl want to identify in these various bodily tissues? The donor—long dead but for a transplanted organ pulsing along in a foreign body. Starzl found what he was looking for. A woman who had undergone a successful liver transplant years ago, for example, had the cells of the donor throughout her body, not just in her secondhand liver. A population of the donor's white blood cells had "hitchhiked" on the transplanted organ and migrated to her hands, her heart, her lungs. These migratory hitchhikers had not met with destruction. They were quietly swimming in her bloodstream and nestling into her internal organs.

Not everyone in the fields of immunology and transplantation accepted what Starzl had found. It went against the accepted understanding of organ transplants: The body would never accept an organ that did not come from an identical twin, so the immune system must be suppressed indefinitely.

Starzl described what he saw as "chimerism." In ancient Greek mythology, the chimera was a fantastical creature with the body of a lion, the head of a goat, and the tail of a serpent. Starzl's patients exhibited microchimerism, harboring living cells that had originated with two individuals.

He pointed out that chimerism had been witnessed for decades in successful bone marrow transplants.

In cases of leukemia, for example, the patient's marrow and blood cells—including

the white blood cells that are the workhorses of the immune system—are wiped out with radiation and replaced by cells produced by the donor's transplanted bone marrow. When the new cells circulate throughout the body and take up the work of the recipient's immune system, the patient is a chimera. Starzl suggested that successful organ transplantation fit the same paradigm, though they had been thought of as different phenomena for decades.

Before he became scientific director of the Thomas E. Starzl Transplantation Institute and before he'd even met Starzl, Fadi Lakkis had heard about his theory.

"But my view of chimerism," says the Pitt professor of surgery and of immunology, "was like the view of most people out there—skepticism, because this Tom Starzl is famous for pushing the envelope to a limit that most mortals are not willing to go to. And like everybody else, and being naïve at the time, I thought, 'This is very interesting,'… but I never really thought about it seriously because the view out there was—I'll tell you honestly—*that's Tom Starzl again, trying to say something that's not possible.*"

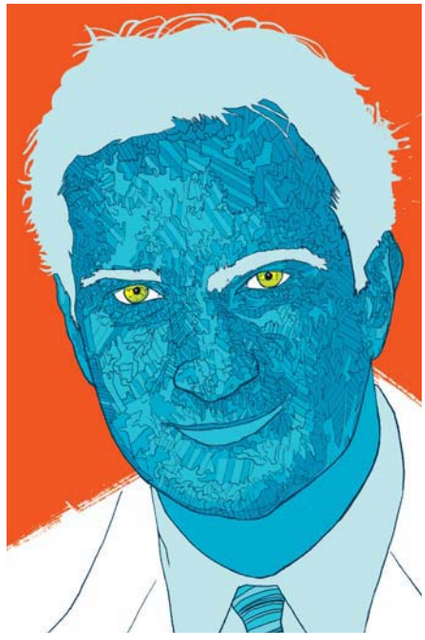
Lakkis points out that this is the same thing the medical community said decades ago when Starzl advocated for liver transplantation—once thought impossible and now an accepted cure for liver disease.

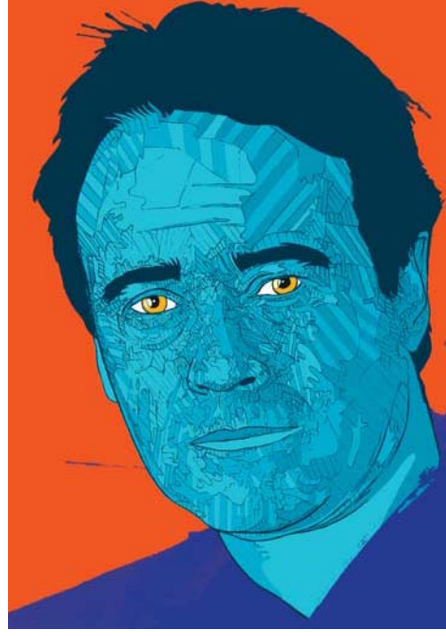
Lakkis, who recently moved to Pitt from Yale University, is still settling into his office at the Starzl Institute. He is tall and friendly, is easy to engage in conversation, and becomes downright animated when discussing how the immune system works or *might* work. (It's humbling to realize how much remains to be discovered about how the immune system works.) Lakkis, an MD, was a practicing clinical nephrologist and director of transplant medicine at Yale, but his affinity has long been for scientists who can happily spend years in the lab investigating the immune system and can pass hours pontificating about its evolution. In the course of his laboratory investigations when he was at Emory University, Lakkis had unwittingly found evidence to support Starzl's theory.

Lakkis first visited Pittsburgh in 1999 to give a talk on his immunology research.

"It was a meeting of the minds," says the once-skeptical Lakkis, explaining that he and Starzl discovered they were thinking about immunology in very similar ways. Lakkis presented research on a molecule produced by the immune system—interleukin-2 (IL-2). It was well known that IL-2 was involved in rejection. When the immune system identifies a foreign invader such as a transplanted organ, IL-2 activates T cells (a type of white blood cell) that recognize and attack the invader. Lakkis and his team experimented with a mouse that did not produce IL-2.

"To our surprise, the organs still rejected," he says.





Two for one: Starzl's successors, Fadi Lakkis (left) and Amadeo Marcos (right), now lead the institute's efforts in research and surgery, respectively.

"So we hypothesized that T-cell activation is required for achieving tolerance. And the reason it is required is it prepares the cell for death. IL-2 activates the cell but also plants the seed of death in it, which makes sense, because you don't want immune responses to keep going and going and going. You want T-cell activation, lymphocyte activation, but you want to ensure that this dies off. … You can get very bad infections as a child, and your lymph nodes can get bigger when you get a sore throat, but then they shrink back. So there are things that regulate the immune system. What we found is that what activates it also regulates it.

"It fit very nicely with what Starzl had been trying to tell the world," says Lakkis. "You've got to let the immune system get activated. Let this battle go on, and that's how you will eventually get tolerance. Instead, in the clinic, people were going around and giving heavy immunosuppression to patients from the get-go, which definitely protects the organs, but then you cannot get off immunosuppression, because once you take them off, those T-cells are still there. So the only chance to get the T-cells to die out is to let that battle happen."

Chimerism, Starzl says, is not just a discovery that cells had migrated from transplanted organs and taken up residence in far corners of the recipients' bodies. The very phenomenon demonstrated why there had to be a mechanism for tolerance.

"We're all exposed to viruses all the time," says Starzl, offering an example. "Eighty-five percent of the population carries antibodies to cytomegalovirus and other hepatitis viruses. I'm swimming in antibodies—you probably are, too—against the B-virus or C-virus." If the immune reaction had as its goal the determination to get rid of every last virus, Starzl says, "85 percent of the world would be dead. So the immune system has found a way to switch the immune reaction off, and that is tolerance."

At Pitt, Lakkis is setting up a lab that will allow him to explore another great mystery of immunology that may help scientists understand tolerance: the innate immune system. In his lab, the research subjects are tiny jellyfish of the genus *Hydractinia*. These are not free-floating jellyfish. In the wild, some species grow on the backs of hermit crabs and are called "snail fur." In Lakkis' lab, they ride glass slides submerged in saltwater.

Jellyfish do not have the highly evolved immune systems of humans and other large animals. They have no lymph nodes, no white blood cells—what immunologists call the adaptive immune system—but they recognize "self" and reject "nonself." When two jellyfish, sponges, or other primitive invertebrates attempt to live side by side on the same surface, they may even begin to fuse together into one larger organism. That's when the battle of the innate immune systems begins. If the individuals are of the same species, and especially if they are related and have genetic similarities, they may eventually tolerate one another and become, essentially, one fused organism with living cells from two—a chimera. The description is reminiscent of what happens when a

mother donates a kidney to her child.

In humans, Lakkis suggests, the innate immune system is like a giant doorbell that can wake the major players of the adaptive immune system.

All sorts of things can ring the bell: inflammation or infection, for example, or a common virus. In a transplant recipient, any event that triggers the innate immune system could initiate a cascade of events that ends with the adaptive immune system vigorously rejecting a transplanted organ. In his jellyfish, Lakkis hopes to answer some fundamental questions that could apply to humans—questions like, what are the mechanisms that turn the innate immune system on and off?

Investigations like these at the Starzl Institute can be applied directly to the hundreds of patients who come here each year for organ transplants.

It's early Tuesday morning on the sixth floor of UPMC Montefiore, and the clinical work of the Starzl Institute is in full swing. At the nurses' station sits a stack of coolers for transporting organs. Beginning as early as 6 a.m., surgeons, nurses, anesthesiologists, and coordinators trickle in, stash their gear, don their caps and masks, and check the white board for their assignments. On a recent day here, surgical teams performed eight transplants: two each of livers, intestines, pancreases, and kidneys. Few other centers have the staff or the space to perform so many in a day.

In a surgical suite down the hall, an organ donor is a few hours into one-half of a living donor liver transplant under the direction of Amadeo Marcos, professor of surgery and clinical director of the Starzl Institute. The recipient will be prepared for the transplant by another team in an adjoining room. In this long, complex procedure, surgeons will remove 60 percent of the donor's perfectly healthy liver as a replacement for the failing liver of the recipient. Marcos pioneered this procedure. He has done more than 300 of these, more than anyone else in the world. At Pitt, around 40 such procedures are performed each year—approximately 25 percent of all liver transplants done here.

The procedure would be almost unrecognizable to someone whose only knowledge of liver transplants was from witnessing Starzl's first attempts in Pittsburgh in 1981. Those operations were done in a crisis atmosphere and required dozens of units of blood. The transplant division created a citywide uproar by draining local blood banks. Today's procedures will be accomplished with no blood transfusions at all.

Starzl and Marcos believe that they are close to achieving tolerance systematically in these patients.

"Living donation creates the scenario for tolerance to occur," says Marcos. "We're in the process of a breakthrough protocol for tolerance. We are still too early in the process to talk to you about results, but it will change transplantation. The patients will not require immunosuppression."

Starzl qualifies, saying that a small percentage of patients will remain on medication, but at such a low frequency—once per week, for example—that it will be nearly the same as being drug free. The side effects at this dosage, he says, are minuscule or impossible to detect.

The living donor procedure, with very few exceptions, usually occurs between blood relatives. Genetic similarities between the donor and the recipient make it easier to achieve tolerance, and the two patients submit to a battery of tests to determine whether they match well in terms of size, blood type, and tissue type. An added benefit of the living donor procedure is that the recipient can be prepared to receive this foreign tissue beginning days or weeks before the actual surgery—something rarely possible with an organ from a donor who may have died suddenly and unexpectedly.

Marcos is cagey about providing details, but the gist of the protocol is this: The recipient is exposed to cells of the donor before the transplant. The immune reaction that is the inevitable—and perhaps necessary—result of transplant begins before and continues after transplantation. Marcos and his colleagues carefully dampen the immune response with steroids and immunosuppressive drugs like tacrolimus so that rejection does not get out of control.

Starzl, who turned 80 this year, continues to quietly move between the lab and the clinic as director emeritus of the institute. His long experience tells him that transplant recipients must go through a bout of rejection in order to reach tolerance. The immune system must be allowed to mount an attack against the foreign tissue and then to become exhausted. The end result, he says, is tolerance, with donor cells being accepted as self throughout the recipient's body.

With his colleagues, Starzl has been praised for showing that transplant patients can have excellent short-term success on relatively modest amounts of tacrolimus—though organ rejection occurs, it is easily reversed. However, the success of a transplant must be measured in decades. Anthony Monaco of Harvard University and Peter Morris of the Royal College of Surgeons of England cautioned in a 2004 forum in the journal *Transplantation* that: "the high incidence of rejection reactions with this strategy may presage late organ injury or loss. Only time will tell. It is incumbent on the authors to provide long-term follow-up of these patients."

Starzl and his colleagues have found that the timing and the dosage of immunosuppression are important at the outset. This evidence may help to explain why Starzl's patients in the 1960s were more likely to achieve tolerance than later patients. The early patients suffered through worse bouts of rejection and then recovered. The later patients received their transplants in the age of advanced immunosuppression, when doctors had gotten very good at preventing rejection. Patients did better in the immediate postoperative period, but because their immune systems had not been allowed to mount a vigorous attack and become exhausted, there was always a possibility of a serious rejection episode, especially if they went off their meds.

Achieving tolerance systematically in a large group of patients would be a breakthrough in both clinical transplantation and in immunology. Starzl is eager to play down his role in these attempts, but Marcos and Lakkis consistently credit him for his regular input and his unusual insight into the mystery that is immunology. (According to the Institute for Scientific Information, Starzl once averaged one paper every 7.3 days, making him one of the most prolific scientists in the world. In 1999, ISI identified Starzl as the most-cited scientist in clinical medicine.)

"He has an unbelievable knowledge of the science of immunology," says Lakkis. "He doesn't want to be the person who can tell you about every single molecule involved in rejection, even though he knows them. He's more interested in the big picture."

"My predecessor, John Fung, and I have just been followers of his ideas," says Marcos."I cannot imagine what we would be without him. I hope that when the time comes that we are somehow ready, but there will never be another Dr. Starzl. Ever. I can tell you that." ■