Fadi Lakkis, an MD and scientific director of the Thomas E. Starzl Transplantation Institute, appreciates the elegance of simplicity. He has an affinity for the simpler question. He savor a good, clean, simple answer. One summer, before he started medical school at the American University of Beirut, he spent his time reading several books on immunology. One of the books was extremely well and simply written, he remembers. “That attracted my attention that someone can explain things in a very simple way,” he says. “It turned out to be quite exciting.”

As the young man progressed through his medical education, the intricacies of kidney disease also captured his imagination, again for the straightforwardness of the physiology. “I found that in nephrology you can diagnose a problem just by understanding the science behind it,” he says. “It turned out to be quite exciting.”

REJECTION RECONSIDERED

Frankly, a lot of people are going to give a damn about new findings by Fadi Lakkis and colleagues. It turns out that organ rejection in transplantation doesn’t happen for the reasons scientists had assumed.

This page: Newly harnessed imaging technology shows that migration of T cells (bright yellow) into transplanted kidney tissue depends on the presence of what’s called a cognate antigen, rather than chemokines. Researchers can watch the process unfolding in living animals. (Blood vessels are green; epithelium is blue; urine is orange.)
Finding a way to achieve tolerance is a lofty goal for many people. For transplant immunologists, it’s the quest of a lifetime. Many a transplant scientist has spent a career looking for a way for the human body to accept an organ without having to resort to immunosuppressive medication.

That’s not to say that contemporary immunosuppressive medication hasn’t been a godsend. It’s allowed for countless successful transplants, legions of lives saved. And over the years the regimen has been finessed, most notably by Pitt’s Thomas E. Starzl. Starzl developed a two-pronged immunosuppressive approach that reduces the amount of drugs a transplant patient takes. Even at the minimum effective dosage, though, the side effects can be unpleasant—and a suppressed immune system lacks the basic ammunition to fight off opportunistic infections and other attacks on the body, such as malignancies.

There are some reports of patients, a handful, becoming tolerant of grafted organs on their own. In other cases, bone marrow transplants have convinced the immune system to halt the attack on the organ. “It’s a little bit drastic,” Lakkis says of that approach. Patients have to undergo chemotherapy or radiation to eliminate their own bone marrow, which leaves them at great risk for infection until the donor bone marrow starts to kick in. “It’s a bit too much for someone coming in for a kidney transplant,” says Lakkis, especially knowing that the immunosuppressive medications are a feasible, if not perfect, course of action.

So the search for tolerance continues. A few years ago, Lakkis decided to go about it from a different angle. “When something has been resistant to good solutions for so many years,” he says, “you start worrying a bit that you’ve been missing something.” He decided to question the fundamental mechanisms of rejection—starting with a paradigm that has been accepted for the past 25 years.

“Organ rejection may seem quite complex,” he says. “In reality, it’s dependent on a single cell type—without that cell type, rejection will not happen. That cell is the T cell. If you take an animal or human that does not have T cells, they will not reject.” The T cell is a lymphocyte, a type of white blood cell originating in the thymus (hence the “T”). It has to get activated—prepared for duty—before it can go to the transplanted organ and initiate rejection. Some T cells are memory cells; they’re already primed by past infections or vaccinations to fight the foreign tissue. Other T cells are naïve and have to be turned into effector T cells before they’re ready to go up against what they perceive to be the enemy—the grafted tissue.

Lakkis was interested in taking another look at exactly how the activated T cells got to the graft. The paradigm involved chemokines—a flexible set of small proteins that can handily fold themselves up and pass through from one side of a membrane to the other. When tissue is inflamed, certain chemokines are present in droves. And a transplanted organ will inevitably result in lots of inflammation, particularly in the delicate endothelium lining of blood vessels.

The long-held assumption was that the crowd of chemokines signaled the T cells to get their attention. An inflamed endothelium is a sticky place. The T cells would slowly roll through the endothelium to the chemokines. Once they met up, receptors on the T cells would bind to the chemokines. With the T cell firmly adhered to the chemokine, the T cells slide smoothly through the barrier of the endothelium and into the grafted tissue where the T cells can initiate the rejection process. You can see how it would follow that if you blocked the chemokines from signaling, you would stop the rejection process. However, attempts to do that had been unsuccessful.

Lakkis decided that he would put his lab to work testing that assumption about chemokines. “We, as a group, enjoy asking these very simple, fundamental questions—the wheres and whys and hows,” he says. “You can come in with very little baggage—just rid yourself of all assumptions.”

This is about when Jeffrey Walch showed up. Walch is an MD/PhD student in the School of Medicine who knows how to make the most of every minute. (He fit the interview for this story into 10- and 15-minute snippets of time between exams and meetings one afternoon.) In 2007, he had been visiting labs, looking for one that would be a good fit for his doctoral work. He and Lakkis hit it off, and he was taken on. His main project for his PhD would attempt to show exactly how those little chemokines direct the migration of T cells to transplanted organs.

“Fadi had some preliminary data from previous work he had done that was suggesting a particular chemokine receptor called CXCR3 was responsible for directing the cells to the graft that would lead to rejection. When I got to the project, that’s where we were,” Walch says.

The CXCR3 receptor is highly expressed, meaning that it’s found a lot on T cells that are activated. At the same time, it’s not found at all on naïve T cells that have not been activated. “It made sense that that had to be the signal,” says Lakkis. “We did a very simple experiment. We took cells that do not express CXCR3, put them in the animal.” To everyone’s surprise, the T cells were able to go in and reject the graft anyway.

Hmmm. What was wrong with this picture? The researchers scratched their heads. Maybe it’s another chemokine-receptor pair? There certainly are a lot of them out there. “This is like a jungle, these chemokines. There’s so many of them,” says Lakkis. “We would have to spend years going through and finding out which one of them is important. And maybe it isn’t a single one that is important. Maybe you knock one out and another takes over.”

On the other hand, he thought, maybe we’ve all been wrong for the last quarter century. Maybe the whole process is completely independent of chemokines. He and Walch decided to test that idea, because it certainly was the simpler experiment. There’s a toxin made by the bacterium that causes pertussis, or whooping cough. If you add pertussis toxin to cells, it blocks the chemokine receptor’s signaling ability. They prepared activated T cells, some with pertussis toxin, others without. If the migration of T cells depended on any kind of chemokine receptors, the T cells treated with pertussis toxin wouldn’t be able to reach the graft.

But they still did. The migration of T cells into grafted tissue was not, as had been thought for so long, dependent on chemokines. The notion was revolutionary.

“At this point, we had deviated from where
the project was initially headed,” Walch says. He changed his dissertation hypothesis regarding how T cells migrate to the grafts. “Because now we didn’t really know—what we thought was working wasn’t working.” For months, Walch pored over the literature from other fields, not just transplantation. He read about different sorts of immune disorders and the central nervous system. When he came across theories that something called a cognate antigen could direct cells, something clicked.

An antigen, of course, is a foreign molecule that revs up an immune response in the body. Experienced T cells are attracted to specific cognate antigens—just as a magnet is attracted to a piece of iron but won’t pick up plastic.

While the accepted paradigm had been that T cells are scattered throughout the body and migrate to different areas when chemokines signaled to them, Walch’s new hypothesis suggested a much more efficient system. “It doesn’t matter if you have a bunch of effector T cells if they can’t interact with the cell that expresses the antigen because they don’t respond to that antigen. Then they’re just sort of a bystander cell that is in the area because there’s inflammation,” says Walch. “But if they’re going where their cognate antigen is expressed,

’ALLO. IS THAT YOU?

Could a marine organism that lives in the shallows of the North Atlantic shed light on the rejection process of transplanted organs in humans? That’s what Pitt’s Matthew Nicotra hopes.

Nicotra, assistant professor of surgery, runs the Hydractinia lab at the Starzl Institute. The particular species he studies, Hydractinia symbiolongcarp—pus, is a colonial saltwater animal—like a coral or sea sponge—that grows until it has covered whatever surface it’s attached to. From time to time, Hydractinia colonies will bump into one another. When that happens, the Hydractinia will either fuse with the others if it senses a good match, or it will fight. But the organism has no eyes, no ears, no nose. How does it know a good match when it doesn’t see one?

There’s an ancient mechanism at work here called allorecognition—the ability to distinguish between self and nonself, which has been observed in all colonial marine invertebrates. “That decision to fuse or fight is controlled, in all [of these colonizing] organisms that have been studied so far, by genetic systems that have very diverse genes as the basis for distinguishing self from nonself,” says Nicotra. “And that is sort of analogous to what happens in human transplantation. If you take an organ and put it into a recipient, there are proteins on that organ that are different between people. And those are basically what the recipient’s immune system recognizes as nonself, and that triggers the immune response.”

Nicotra wondered, does the Hydractinia’s mechanism of allorecognition share an evolutionary history with the human’s innate immune system—the first line of defense against pathogens? So far, his lab has identified the genes that encode the various proteins that are analogous to certain cell-surface molecules called the major histocompatibility complex (MHC) in humans. “Those don’t appear to be similar, but that’s not surprising because those are exactly the proteins that evolution, over at least 500 million years, has been making diverse,” Nicotra says. “Five hundred million years is probably a lowball estimate of the divergence time between Hydractinia and humans.” The more likely route to answer this question, he says, is to look at the signaling pathways that control allorecognition in the sea creature and compare those pathways to those involved in transplant rejection. Stay tuned as Nicotra’s lab continues to explore the depths of the immune response. —SF
then they're going where they need to go.”

What an idea, Walch thought. Activated T cells would only go to where they needed to be.

To test the hypothesis, they put to use a new technology. In 2007, Lakki had recruited researcher Geoffrey Camirand to the Starzl Institute. Camirand, whose work on research interests include studying a subset of T cells called regulatory T cells, worked with Lakkis to set up an intravital imaging system that would be able to look at what is going on inside a living animal. The new system made use of two-photon microscopy. Although the intravital two-photon technology had been available since 2001, the bulk of the research employing it had been looking at how the immune system operates in lymph nodes. Not much had been done using it to study transplants.

To understand why everyone was so excited about the new imaging system, you have to understand several components. First, the two-photon system was the answer to the problem of the single-photon laser, which is preferable when a higher resolution is needed, for instance, in the imaging of cell organelles. Single-photon technology can make beautiful pictures but can’t penetrate deeply, because of the elevated scattering of the high-energy photons within tissues. It can also cause tissue damage. In two-photon microscopy, the photons are concentrated into a single focal point. The point where they meet with a fluorescent molecule is the only point that shines. “And it shines brilliantly,” Lakkis says. “In that 1-micron focal plane where they meet, you can see the cell that is shining. It emits beautiful fluorescence.” (See p. 13.) There’s an awestruck note in his voice as he relates this.

“You can study the living tissue. It’s almost like four-dimensional imaging, because you can tell what it’s doing [for] up to 2-and-a-half hours. This is a more definitive technology.”

“It’s good that we jumped on this technology a few years back,” says Camirand, “because now we can apply it to a wide range of transplant-related questions. There’s a huge amount of work that can be done with this technology.”

They set about testing Walch’s hypothesis: Lymphocytes, like T cells, have receptors on them that are very specific to what they are supposed to recognize. “Usually it’s a virus or bacteria. But in the setting of a transplant, the antigen could be almost any foreign protein on the [transplanted tissue] that is different from the tissues of the recipient,” says Lakkis. The transplanted organ expresses proteins—antigens—that are different from the recipient’s because no two people’s organs are alike in this way—unless they’re identical twins. “We did simple experiments where the T cells are very specific to one antigen, and the transplanted organ has that antigen or it doesn’t. And we found that only if the antigen is present do the T cells get in. If the antigen is not present, the T cells cannot get into the tissue of the organ and reject it.” As Walch had hypothesized, the migration of T cells into the bloodstream and bring them over to the other side. That is a completely new paradigm, as well.

With Walch as the lead author, the work was published this May (online, appearing in print in June) in The Journal of Clinical Investigation. Terry Strom, professor of medicine and surgery at Harvard Medical School and co-scientific director of the Transplant Institute at Beth Israel Deaconess Medical Center, published a commentary of the work, titled, “Transplant Rejection and Paradigms Lost,” in the same journal. “Dr. Lakkis’ work has revolutionized our understanding of the inception of transplant rejection,” he says. “Several widely held views were disproven and the work should influence attempts to prevent rejection.”

While the therapeutic implications are exciting, Lakkis says he tends to be more reserved about them. It takes a long time to translate newfound biological understanding into a treatment for patients.

Walch, PhD in hand, is completing his last two years of med school. When he’s done, he hopes to be a plastic surgeon, specializing in cleft lip and palate while continuing to do research in immunology.

Lakkis continues to go back to the fun-
About a decade ago, he took a sabbatical in Leo Buss’ evolutionary biology lab at Yale University where he collaborated with then-doctoral student Matthew Nicotra. (See sidebar p. 15.) Lakkis was fascinated by the allorecognition response being studied on a sea creature called Hydractinia. (Allorecognition refers to an organism’s ability to tell the difference between self and nonself cells or tissues of another member of the same species.)

“The ‘primitive’ organisms do not have lymphocytes, and yet they are quite good at detecting and rejecting tissues of an unrelated organism,” says Lakkis. Lymphocytes are the hallmark of the adaptive immune system, which has been primed to recognize and attack foreign antigens by previous contact with them. “This told us that there must be nonself tissue recognition mechanisms in mammals that predate lymphocytes, which was not what everybody else thought.” In a recently submitted paper, Lakkis and his team investigated the innate immune system of mice, which is made up of cells other than lymphocytes, and found that it indeed distinguishes tissue that is foreign from self.

Questions beget more questions: How do the innate and the adaptive immune systems work together to reject a transplanted organ? What are the earliest events that lead to graft rejection?

Notes Lakkis, “We’ve found that there is almost never a fundamental question that is not only worth visiting, but also worth revisiting.”

ROLES AND REGULATIONS

Angus Thomson, Distinguished Professor of Surgery and Immunology at the Starzl Institute, speaks in a soft Scottish burr. The PhD could be telling a bedtime story, his words roll out so smoothly. But the story he is relating today has to do with regulatory immune cells, which he has invested a large part of his career in studying. And it’s an exciting story because regulatory immune cells just may be a key to keeping transplanted organs from going through rejection.

It’s important for immune cells to fight the good fight against pathogens. But it’s just as important for them to know when to stop. That’s one of the things regulatory immune cells do—tell the immune system to step aside. So transplant immunologists like Thomson have long been wondering if regulatory cells could be put into service to suppress the immune response in transplantations.

There’s encouraging news on that front. In a recent National Institutes of Health–funded study using a nonhuman primate model, Thomson investigated whether a certain kind of immune cell called a dendritic cell could prolong the survival of a transplanted organ. Dendritic cells have a gnarled-looking structure with appendages jutting from them like knobby tree branches. They help to regulate the immune system by either calling T cells into action or by suppressing their response. For this research, dendritic cells were taken from the blood of rhesus macaques that would later be the donors of the transplanted organ. The cells were treated to promote their ability to negatively regulate immune response and then infused into the recipient prior to the transplant. The monkeys that were not given the infusion lasted 40 days before rejecting the transplanted kidney; yet those who were given the dendritic cell infusion survived 113 days before rejection. “With the simple expedient of infusing these immune cells a week before transplant, we were able to prolong transplant survival,” says Thomson. “This has never been shown before in a preclinical model. The data suggest it may well be worthwhile moving forward to design a human trial in kidney transplantation using this type of regulatory immune cell.” Thomson imagines this approach might help transplant patients use less potent immunosuppressive regimens.

The study was published in this June’s online edition in the American Journal of Transplantation. Mohamed Ezzelarab, research assistant professor of surgery, is lead author. —SF