Seeking Clues to Transplantation Tolerance

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When Dr. Thomas Starzl pioneered pediatric liver transplants in the late 1960s at the University of Colorado, the procedure was controversial, as many children rejected their transplanted organs and died. More kids started living longer as surgical techniques and antirejection drugs improved. While at the University of Pittsburgh Medical Center, Starzl and colleagues tested cyclosporine, an immune suppressant developed from a fungus, that radically reduced acute organ rejection. A decade later, they started using tacrolimus (FK506), also fungus derived, which was up to 100 times more powerful than cyclosporine but with fewer side effects.

Like Odysseus deciding whether it was better to have his sailors eaten or lose his boat in the whirlpool when squeezed between the monsters Scylla and Charybdis, organ transplant recipients still face unpleasant tradeoffs. Closely matched donor organs are scarce; long-term use of immunosuppressant drugs causes side effects and can lead to infections and cancer.

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Over the years, however, Starzl and other transplant researchers noticed that certain patients seemed to exist in an immunological state of grace. They went off immunosuppressants for various reasons but didn't reject their transplanted organs. Indeed, in studies of selected groups of patients who received organs from live donors, around 1 in 5 could stop taking immunosuppressants altogether or dramatically curtail their dosing with no ill effect (Mazariegos et al., 1997; Margolis and Turka, 2008).

The more closely an organ is HLA (human leukocyte antigen) matched, the better the chance of tolerance. Organs from live donors, such as parents or siblings, are better tolerated than those from random deceased donors. Organ condition and age matter as well.

Researchers want to be able to predict tolerance, but no biomarkers exist to indicate who will tolerate grafts. What sets the naturally tolerant apart? Is it possible to train the immune system to selectively recognize an allograft without mounting an inflammatory response?

Outgrowing Rescue Therapies

The concept of "immunological tolerance" can be traced back to the 1950s and the skin graft work of Rupert Billingham, Leslie Brent, and Sir Peter Medawar (who received a Nobel Prize in 1960 for his work with Sir Frank Macfarlane Burnet.) Yet the field of tolerance in transplantation is still inching its way forward. Better tools and animal data promise progress. But a driving force is that kids are growing up with transplanted organs.

Dr. George Mazariegos, director of Children's Hillman Center for Pediatric Transplantation and the Thomas E. Starzl Transplantation Institute, notes that while pediatric organ transplantation used to be focused on "rescue therapies" to achieve 5 year survival, at this point, 5000 children in the U.S. are living with transplants. "Now we are seeing late-term morbidities," said Mazariegos. While transplanted organs in children can now function for their lifespan, immunosuppression affects growth, blood pressure, kidney function, and tumor risk. "If we can lower it in children or eliminate it, it would be a dramatic advance," said Mazariegos.

Liver transplantation patients seem to achieve better tolerance, but only 1% of pediatric liver patients can go off immunosuppressants entirely, Mazariegos estimates. About 50 liver transplant patients at Children's Hospital have gone off immunosuppressant drugs completely. Others, after being initially stabilized, are brought down to the lowest possible dose; however, children who have HCV or autoimmune disease are not good candidates for drug-weaning.

Building a Research Network

The UCSF-based Immune Tolerance Network (ITN) (http://www.immunetolerance.org) sponsors research groups around the world that are developing predictive assays using genetic and immunological markers as well as running trials using different approaches to induce selective tolerance. They hope to improve long-term outcomes in organ transplants and pave the way for better treatments for T cell-mediated autoimmune conditions, including type 1 diabetes, lupus, asthma, and allergies. Founded in 1999 and directed by Dr. Jeffrey Bluestone, the Immune Tolerance Network currently comprises around 40 institutions and is supported by a $160 million, 7 year contract with the National Institute for Allergy and Infectious Diseases, the National Institute for Diabetes and Digestive and Kidney Diseases, and the Juvenile Diabetes Research Foundation.

According to Vicki Seyfert-Margolis, Ph.D., ITN chief scientific officer, the ITN generally sponsors therapies in early phase II with proof of safety and animal efficacy data that at least indicates biological mechanisms relating to tolerance. So far, therapies have focused primarily on T cells; most are targeted at the process involved in T cell activation or through T cell depletion. Some antibody therapies such as nonmitogenic anti-CD3s and CTLA4Ig are seeing dual use for transplant and autoimmune conditions. Additionally, attention is being paid to B cells, resulting in a study using rituxumab for depletion of B cells in mice as well as to an exploration of alloantibodies (which develop in the recipient in response to grafts and spur rejection) and combining T cell-targeted therapies with rituximab. "I'd not say that anything has emerged as a clear winner here," said Seyfert-Margolis.

Advertising for People Who Ditched Their Meds

Although the ITN advises kidney transplant recipients not to stop immunosuppressant medications without medical supervision, they are actively looking for people who, for whatever reason, managed to do just that and keep their transplanted organs. Dr. Kenneth Newell, associate professor of surgery at Emory University, is compiling the ITN Registry of Tolerant Kidney Transplant Patients. The U.S. registry collects blood and tissue samples looking for genetic and immunological insight into what makes their immune systems unique. Finding the innately tolerant takes ingenuity: one productive strategy was an advertisement in Alaska Airlines magazine. So far, the registry has enrolled 25 people and Newell's study has enrolled 171 participants, including those in additional comparison study groups.

The ITN is currently sponsoring 12 active studies and several more in development. One example is an ongoing prospective phase II trial under the supervision of Dr. Avraham Shaked, director of the PENN Transplantation Center, which involves weaning a projected 275 adults with liver transplants (some with HCV) from medication. The study is comparing immune and genetic profiles of HCV-infected transplant recipients as well as examining the use of T cell depletion with Campath-1H (alemtuzumab, a humanized anti-CD52 monoclonal antibody). Another multicenter study is weaning adult kidney transplant recipients off immunosuppressants.

ITN-sponsored trials also include studies in mixed chimerism in kidney transplants (David Sachs and Benedict Cosimi, as well as Megan Sykes at Massachusetts General Hospital), use of Campath-1H/Rapamycin in kidney transplantation (Stuart Knechtle at the University of Wisconsin Medical School), and a kidney transplant weaning trial of LEA29Y (Flavio Vincenti, University of California, San Francisco, and Christian Larsen at the Emory Transplant Center).

"Costimulation blockade implies blocking those molecular pathways that are important in instigating T cell-mediated immune responses," Thomson said. "Such strategies have proved highly effective in small animal models, not so effective in nonhuman primates." Thomson said costimulation blockade is still promising as an adjunctive immunosuppressive therapy. The CD40/CD40 ligand pathway has received the most attention, but initial clinical testing of an anti-CD40 ligand monoclonal antibody was curtailed because of blood clots.

One technique is to deplete patients' immune systems before transplantation in order to minimize immunosuppressants afterwards. At Pittsburgh, researchers use Campath 1H to deplete peripheral T cells and B cells in pediatric kidney patients prior to transplantation, then lower the dose of immunosuppressants afterwards.

Creating Chimeras

Another technique that is closer to achieving true tolerance is inducing "mixed chimerism" based on blending donor and recipient immune systems. In January 2008, Dr. David Sachs and colleagues at Massachusetts General Hospital reported results of a trial of 5 kidney transplant patients between 22 and 46 years of age. Their kidneys came from parents or siblings but were immunologically mismatched. Sachs' team ablated their immune systems with radiation and chemotherapy and depleted their T cells with drugs. They then transplanted the donor kidneys along with an infusion of bone marrow cells isolated from the recipient. "Over the first few weeks the patients are rather immunosuppressed, but when their immune system does come back, it comes back tolerant to the recipient as well as to the donor, but not to anything else," said Sachs. "Not to third party. Not to bacteria. Not to viruses. So you are left with a much better immune system than you would have on immunosuppressive drugs." The doctors stopped the drugs after 9–12 months. Two-to-five years later, four patients still didn't need immunosuppressants. The fifth patient rejected the kidney, possibly sensitized by prior exposure. He successfully received a second kidney transplant on standard immunosuppression.

"What you are doing is trading an upfront more difficult and potentially dangerous procedure for a lifetime not having to be on immunosuppressive drugs," Sachs said. "Even if patients take their drugs properly, we haven't been able to stop chronic rejection." According to Sachs, chronic rejection claims about 5% of organ transplants a year. "We are hoping that tolerance will avoid that as well," Sachs said. According to Sachs, in their animal studies, monkeys have not shown chronic rejection in 10 years. The next steps are to reproduce these results in more patients and to study the use of unrelated donors and, hopefully, deceased donors.

Inhabiting an Immunological State of Grace

Amy Whitacre, an ebullient 29 year old architect in Ohio, lives in a state of immunologic grace. She is the elusive key to the mystery of tolerance. When Whitacre was 6 months old, her parents noticed something was wrong. They took their baby from doctor to doctor. Eventually, she was diagnosed with idiopathic cirrhosis of the liver. Her liver swelled pathologically, putting pressure on her aorta and creating varices—abnormally enlarged veins in the lower part of her esophagus. Doctors feared they would rupture and she would bleed to death. Whitacre recalled an otherwise normal childhood, but she couldn't go out and play because she had to take phenobarbitol at night.

In 1987, when she turned 8 and before her liver failed, Dr. Thomas Starzl transplanted a new liver into her at Children's Hospital of Pittsburgh. "I've been very lucky and never went into any sort of rejection," Whitacre said. As she grew, her doses of cyclosporine, antibiotics, and steroids became more infrequent. "It got to the point that I was taking my meds on Tuesdays and Thursdays my last year in college," Whitacre said. Her doctors (Mazariegos among them) biopsied her liver and periodically checked her blood. Whitacre has been immunosuppressant free for 7 years. She periodically participates in studies run by Mazariegos. "I hope there is something about me that can help others," Whitacre said.

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