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Presentations

Pediatric Transplant: Current and Future Challenges for Future Improvement

Speaker: Dr. William Harmon, Director, Division of Nephrology, Children's Hospital of Boston, Harvard Medical School.

End stage renal disease (ESRD) is uncommon in children and the incidence has remained about the same in the past 30 years. According to Dr. Harmon, each year approximately 1,000 children develop ESRD and 600 to 700 children receive kidney transplants. More than half of all children who receive kidney transplants are older teenagers; about a quarter are children ages 10 to 14; and about a quarter are children under age 10. In the youngest age groups, most children who need transplants are males with congenital obstructive uropathies. In older age groups, gender differences tend to disappear, but racial differences increase, with a greater percentage of African American children receiving transplants than are represented in the total U.S. population.

While in adults, the main causes of ESRD are diabetes and hypertension, in children, the main causes are congenital and familial inherited disorders, Dr. Harmon said. Therefore, the care of children is different than the care of adults. Most children diagnosed with ESRD have had it for their entire lives and many require reconstructive surgery before they receive a transplant. The most common acquired disease in children with ESRD is focal glomerulosclerosis, a disease with a high recurrence rate after transplant.

Although young children were previously thought to be high-risk kidney transplant recipients because of the intensity of their immune responses, current studies show that they have low rates of acute rejection. In addition, they also have the best long-term graft survival rates of all age groups, including adults. Adolescents on the other hand have the worst graft survival results except for adults older than age 65. One of the reasons for this may be lack of compliance with drug protocols, particularly with steroids, which cause cosmetic side effects. Graft survival in adolescents is an area that needs further study.

Most children who receive kidney transplants receive them from living donors. In 2001, 57 percent of children with kidney transplants received them from living donors compared with 43 percent in 1987. Recent studies have shown that all children who receive living donor transplants do better than those who receive cadaver transplants. Other advantages to living donor grafts include the ability to schedule and the use of tolerance induction protocols.

Cooperative Studies and Registries

Dr. Harmon described two cooperative studies of pediatric transplantation and described their functions:

- **North American Pediatric Renal Transplant Cooperative Study (NAPRTCS).** Established 15 years ago, NAPRTCS is a voluntary cooperative with 147 actively participating centers and four registries—transplant, dialysis, chronic renal insufficiency, and FSGS. So far, the study has enrolled almost 13,000 children in every phase of the treatment and has conducted 39 special studies. The collaborative has collected important information on transplants in children and helped to define the focus of future studies.

- **Cooperative Clinical Trials in Pediatric Transplantation (CCTPT).** The cooperative has been in existence for nine years and has conducted six trials, with two more scheduled for fall 2003. Major studies include research on induction antibodies, steroid withdrawal, calcineurin inhibitor avoidance, varicella vaccine, school performance of children with transplants, immunoglobulin for highly sensitized patients, and mechanistic studies, which are a marriage of basic science studies and clinical trials. Early results of the cooperative's mechanistic studies have shown up-regulation of cytokine genes in renal graft biopsies, a finding that has extended the study to urine and blood samples in an effort to find markers of early graft rejection. In the next few years, the cooperative will concentrate on enrolling larger numbers of patients in longer trials. Currently, the study is enrolling 100 to 150 children per year, which is approximately 20 percent of the total number who receive transplants.

Improving Pediatric Kidney Transplantation— Challenges for the Future

Dr. Harmon outlined the following major challenges for future improvements in pediatric kidney transplantation:

- **Graft thrombosis and chronic graft rejection.** Graft thrombosis is currently the major cause of graft loss in children. Why this occurs and how to avoid it needs to be determined. Chronic graft rejection is another major cause, and although it can occur in all transplanted organs, the kidney is particularly vulnerable. In this condition, blood vessels supplying the transplant eventually become occluded and the graft is lost because of lack of perfusion. Rejection is usually determined after it occurs. Determining the early stages of chronic graft rejection is a priority for transplant research.

- **Chronic medication toxicity from immunosuppression.** In patients who receive immunosuppressive drugs, the incidence of tumors and bacterial and viral infections, especially post-transplant lymphoproliferative disorder (PTLD), are increasing and replacing acute rejection as a cause of hospitalization. The

incidence of PTLD, which is caused by the Epstein-Barr virus, is greater in children than in adults. Little is known about prophylaxis, value of monitoring, preemptive treatment, definitive treatment, and which immunosuppressive drugs should be used after PTLD infection occurs.

- **Immuno-responsiveness assessment.** The choices of how to use immunosuppressive drugs are obscure, and physicians do not know which individual patients or classes of patients should receive them. Tests that assess the immuno-responsiveness of a patient would assist physicians in making decisions concerning which drugs to use and what doses are appropriate. Ideal pediatric immunosuppression would be effective, would be given infrequently, would have no cosmetic side effects, and would be easily adapted to individual responses.

- **Fine-tuning tolerance induction protocols.** Because acute rejection episodes set up a cascade of chronic graft rejection, overdoing a tolerance protocol can trigger this cascade.

- **Drug minimization protocols and development of new drugs.** In the next 10 years, the use of steroids and calcineurin inhibitors as immunosuppressive agents for children who have received transplants will most likely decrease. Steroids cause multiple serious side effects, including diabetes, growth failure, cosmetic problems, osteoporosis, and premature cardiovascular consequences. Calcineurin inhibitors inevitably cause nephrotoxicity, which in 10 percent of graft recipients leads to ESRD. Hypertension, hyperlipidemia, and cosmetic problems are additional side effects of calcineurin inhibitors. CCTPT plans to conduct a multi-center, randomized study of a steroid-free protocol and a pilot study of drugs that produce co-stimulation blockade. This pilot may lead to a larger study of calcineurin inhibitor and steroid avoidance. Only a few drugs are currently in Phase II trials. Probably the most exciting Phase II research is on co-stimulation blockade antibodies. Drug research will focus on individualization and minimization of immunosuppressive drug use and on tolerance.

- **Racial disparities in graft survival.** After the first few years post-transplant, African Americans of all age groups have a steeper decline in graft survival rates than people of other racial and ethnic groups. The Secretary's Advisory Council on Transplantation has advised that the causes of this should be a focus of future RFAs issued by the NIH.

Summary

Dr. Harmon suggested the following important areas for future research studies:

- Mechanistic studies

- Infectious and cardiovascular complications of immunosuppression

- Graft thrombosis
- Chronic allograft nephropathy (chronic graft rejection)
- Proper endpoints for pediatric trials
- Adolescent outcomes
- Recurrent disease
- Racial effects
- Tolerance protocols
- Evaluation of immunotherapy protocols for effectiveness and toxicity
- Cadaver graft survival in children by race

In summary, Dr. Harmon emphasized that most important of all, pediatric trials should not compete with each other for resources, and the resources here are the children.

Questions and Answers

Q: Is the trend in adolescent graft survival rates general for all transplants or is it specific to the kidney?
A: It is general. We have a registry called the Split Registry for Liver Transplants and it shows the same characteristics for adolescents who receive liver transplants. The same is true for heart-lung recipients.

Q: Do hormonal issues related to puberty affect graft survival rates in adolescents?
A: That is an interesting question. My first response, however, is that the drugs used for immunosuppression, particularly steroids, actually delay puberty substantially.

Q: Is it correct that there is no increase in acute rejection in the 11 to 17-year-old group?
A: Yes. The increase in this age group is only in chronic rejection.

Q: Certainly the problem of chronic allograft loss is a tough one because of the lack of surrogate markers. What are your suggestions on how to encourage research on improving intermediate markers?
A: We need to have some biologic markers. There are some quantitative stain trials going on right now of kidney biopsies in which researchers are trying to quantify the amount of fibrosis that occurs, so that when a patient receives treatment to slow down the rejection, you can at least see if the treatment is working.

Q: Are they looking at both the vascular component and the interstitial fibrosis?

A: It's mostly interstitial fibrosis. Looking at the vascular component would be very interesting. I think the heart community is way ahead of us in terms of using coronary artery ultrasounds for their transplants.

Q: How many cases of PTLD are we talking about, and do you have any insights into the decline of PTLD in heart transplants?
A: In kidney transplants the rate is about 1 percent (as opposed to a 50 percent rate in patients with intestine transplants), so one out of a hundred is not alarming. In pediatric trials, the rate is 2 to 3 percent. Trials of our minimization studies are showing a substantially greater PTLD rate than we expected to see, so it probably is the immunosuppression. In terms of PTLD in heart-lung transplant patients, there have been no controlled trials. The heart transplant community used substantially less tacrolimus and more cyclosporin. Probably tacrolimus was a bigger factor mostly because it was more potent, not because there was anything idiosyncratic about it. The approaches to eliminating or treating PTLD are early identification, which is just in its infancy, early treatment with antivirals, which are not very successful with Epstein-Barr virus, and withdrawal of medications. I think the heart transplant community has been a little more aggressive about adjusting medications by doing biopsies and falling back.

Q: Does the risk of PTLD go up with time or is there a window of increased risk?
A: It's occurring much sooner than it used to, so there is no absolute limit. We just treated a 17-year-old girl who had a transplant at age 4. It's probably community-acquired EBV—that's when you get mono and you can't avoid that.

Q: What is your opinion about why there is a decline of graft survival in the African-American population?
A: I don't have any opinion because I don't have much factual data. There are questions about the higher frequency of hypertension in this group. Some of the gene polymorphisms are thought in some way to be a problem and are obviously different in African Americans. There was a question about African Americans receiving more poorly matched grafts. What weighs against that is that the curve is similar in living donor grafts and cadaver donor grafts.

Q: Hispanic survival seems to be better. How does NAPRTCS identify Hispanic?
A: It's not as careful as what NIH does. If it's checked off as Hispanic, it is Hispanic.

Q: Is there an attempt to separate Mexican descent from say Caribbean?
A: No. The socio-economic factors are an important issue. My impression is that in pediatrics we are getting the drugs to them. There has always been the concern that two or three years after transplant is when you lose Medicare coverage and maybe patients aren't getting the drugs, as well. According to our studies, pediatric drugs seem to be prescribed and paid for in one way or another, particularly through state Medicaid programs. I suspect the better survival rate of Hispanics is biological not socioeconomic.

Q: Back to the biomarker issue, does either NAPRTCS have or the CCTPT have samples that were put away that could be used in long-term follow-up studies, and do they have appropriate consent?
A: The consent was appropriate for the times. Yes, they do have samples. In the first induction study, there were about 200 biopsies obtained at various times and in the current steroid withdrawal study there is a 6-month biopsy. In all of the patients in our calcineurin avoidance study, there are four biopsies in the first year, and they're all frozen. RNA analysis is being done, and DNA analysis can be done. Our labs aren't very eager to look at gene chips because I think it's still pretty much a fishing expedition. As to whether or not consent is correct—it will be in the future but it probably is not by today's standards. And even in the future, it's questionable because many of our IRBs say you can't do anything unless you re-approach the patient. If we can't find the patient, it's too bad. We couldn't do the Framingham study today by many of the IRB rules.

Q: What about serum samples?
A: We have serum and urine.

Q: One of the things we see in adult transplantation is the use of intense anti-T-cell therapies Do you think modulating the immune system of a child using Campath-1H or high-dose thymoglobulin would potentially have hope?
A: Some of the depletion strategies are a little scary. I liken it to what was done early on with thoracic duct drainage. It's sort of a nuclear holocaust approach to immunosuppression. In most cases, we have decided not to let children do that until we have lots of data in the adult studies. We almost did a CD-40 ligand study and stopped it very quickly as soon as the adult data were accumulated. For the Campath-1H and thymoglobulin studies, we're going to require a little more time and success in adult studies.

Q: Speaking of new agents, do you have any clinical results on CTL4 antibodies? What kind of side effects are you seeing in your populations? At least in adult studies they seem like ideal candidates because they have minimal side effects.
A: We do have a promise from Bristol-Myers-Squibb hopefully to start clinical trials with LEA29Y in January or February, but there is no preliminary data.

Q: Do the different clinical conditions of the various age groups affect graft survival in these groups? I know you only have about 700 transplants a year, but do you have enough to compare subgroups?
A: Young children have developmental disorders and they often need lots of surgery before their transplant to make sure their urinary tracts are reconstructed appropriately. The good news from that perspective is that they don't have recurrent disease. They have technical issues because we are virtually always putting in an adult kidney, which we have to perfuse. So in infants and young children, we must keep their blood pressure up rather than down. Young children metabolize immunosuppressants much quicker than adults. Rapomycin, for example, was touted as having a half life of about 50 hours in adults; the half life in a child is about 12 hours, so we have to give not only bigger doses, but also much more frequent doses. We learned by doing until we got better studies. As

children get older, they develop other diseases such as FSGS and immunologically mediated diseases, and recurrent disease becomes a bigger issue. I'd like to say that graft thrombosis goes away as children get older because you've gotten rid of the technical complications, but there's another "blip" in the adolescent age group that's totally unexplained at the present time. Metabolism of drugs changes as children get older and their behavior changes. Parents of young children give them their medications; parents of adolescents tell them to take their medications. We can do studies, but the registry is voluntary, and we have to deal with the data we get.

Q: There is a significant disparity on the data slides you have shown between pediatric and adult transplants done each year. Could you give an estimate of what the pediatric recipient pool is and whether there is a similar concern as we have with adults waiting for grafts?
A: We're quite proud of the fact that we've pushed the pediatric issues to the point where UNOS is afraid to say "Oh, this may disadvantage children." We've been loud, vocal advocates for children. Obviously, 55 percent are receiving living donor transplants. Children under 18 years of age are the only group that receives preference on the cadaver donor waiting list because of the decided advantages to early transplant, particularly in terms of growth. Unfortunately the flip side of this issue is that the transplants may be less well matched because children are not in the pool long enough for that to occur.

Tissue Engineering, Stem Cells, and Cloning: Applications for Regenerative Medicine

Speaker: Anthony Atala, M.D., Professor of Surgery, Director, Laboratory for Tissue Engineering and Cellular Therapeutics, Children's Hospital of Boston, Harvard Medical School

Although the first kidney transplant occurred almost 50 years ago, Dr. Atala said, physicians are still dealing with some of the same challenges that result from organ shortage and rejection—tissue loss and the need to replace it with tissues from other areas of the body or from other persons. Because these choices are not always ideal, scientists have begun to look at tissue engineering as a way to develop organs and additional tissues for reconstruction.

Tissue engineering is an outgrowth of the field of cell transplantation, which started in the 1930s. Nobel Laureate Alexis Carrel and Charles Lindbergh, of aviation fame, published the book, *The Culture of Organs*, in 1938. Forty-three years later, in 1981, the first tissue-engineered graft, a skin graft, was performed. However, despite its relatively long history, tissue engineering has made few clinical advances to date. The major impediments to this technology have been the inability to expand cells *in vitro* and the inadequacy of biomaterials on which to grow them.

Growing Cells *In Vitro*

In the past, scientists were able grow cells *in vitro* for approximately two weeks, but the cells then perished. In recent years, scientists have studied growth factor mechanisms and progenitor cell populations, and with this knowledge, they were able to identify cell populations that could be expanded.

In the past 10 years, the Laboratory for Tissue Engineering and Cellular Therapeutics at Boston Children's Hospital and Harvard Medical School has successfully grown and expanded several cell populations, including urothelial cells. By studying bladder injury in live small animals, the scientists determined that the bladder could regenerate in 12 hours. In a study using a small animal bladder injury model, scientists injected the bladder with BrdU, the thymidine analog that is incorporated into the cell's DNA. By doing so, they were able to identify the bladder's progenitor cell population, which they isolated. The cells were then grown in serum-free media with specific additives, and scientists were eventually able to identify the growth factor cytokines that caused the cells to differentiate. By avoiding the growth factor cytokines, they were able to expand the urothelial cells *in vitro*. Today, the lab's scientists can take "a square centimeter of bladder tissue obtained from a biopsy and in six days turn it into enough tissue to cover a football field," Dr. Atala said. "This is all done with normal, primary human cells; no retroviral vectors are used, nor are any other kinds of manipulations employed. The cells retain a normal karyotype after multiple passages."

Biomaterials for Supporting Cell Growth

Scientists at the laboratory isolated specific biomaterials that are compatible with the human body and formed them into scaffolds. They then seeded the scaffolds with cells and observed certain factors such as apoptotic activity, cell proliferative activity, and seeding density of specific cell types. Manmade biomaterials as well as natural biomaterials were studied. The researchers followed one principal, Dr. Atala said; that is, "The optimal biomaterial should be one that replicates the structural, architectural, and biomechanical properties of the tissue or organ you're trying to replace." Because the biomaterial acts as a prosthesis until the cells are able to take over, engineered tissue must withstand the pressure of the organ that is being replaced.

Another problem encountered in tissue engineering is that of transplanting a large volume of cells. In the past 20 years, scientists who attempted to transplant cells discovered that volumes could not be greater than $3mm^3$, which is about the size of a pencil eraser. Larger volumes developed major nutrition and gas exchange problems, Dr. Atala explained. In nature, all organisms solve this problem, that is, grow large volumes of tissue, by branching. With this in mind, Dr. Atala's group developed scaffolds with branching patterns that allow cells to grow as sheaths on top of the scaffold. The biodegradable scaffolds also provide pore sizes that promote angiogenesis and innervation. Vascularization is promoted not only by a 3-D scaffold, Dr. Atala said, but also by delivering growth factors such as vascular endothelial growth factor (VEGF) to

the cells, the scaffolding, and the engineered tissue; by employing encapsulated protein delivery systems; by adding endothelial cells to the constructs; and by adding vascularized tissue around the constructs.

During the mid-1990s, Dr. Atala and his colleagues also learned how to construct a hollow organ; they layered a scaffold with muscles cells and endothelial cells and then rolled the scaffold into a tube. Eventually, they developed an organ with the layers of the bladder as well as other hollow and tubular structures.

Regenerative Medicine—Replacing Tissues and Organs

Engineering tissues that are composed of more than one cell type is a complicated process because the cells must attach at the correct level, Dr. Atala said, and they must all be compatible. Until recently, the only tissues that have been created *in vivo* using athymic mice have been tissues of human single cell types. A breakthrough was made several years ago, when Dr. Atala and colleagues created the first engineered organ, a neo-bladder. Their strategy involved harvesting urothelial and smooth muscle cells from canine bladder biopsy specimens and seeding the cells onto preformed bladder-shaped polymer scaffolds. Smooth muscle cells were layered on one side and urothelial cells on the other. The hollow, urinary bladder was transplanted onto bladder remnants in dogs. The transplants developed normal morphology and functioned as early as one month after surgery.

The Laboratory for Tissue Engineering and Cellular Therapeutics has also developed applications for the urethra and ureters. Several years ago, they developed collagen biomaterial scaffolds that are tissue-specific. When transplanted without cells, the scaffolds heal well, he said, if they are 1 centimeter or less; however, in anything larger, wound healing with the formation of scar tissue occurs. Therefore, larger scaffolds must be seeded with cells. Using these scaffolds and seeding them with autologous cells, Atala and colleagues have created engineered urethras, which they have successfully transplanted into patients who had hypospadias and urethral strictures.

Researchers at the lab used a similar process to construct neo-ureters, which they have transplanted into dogs. The ureter is more complicated to construct than the urethra because it has peristalsis. While the urethra expands and contracts a few times a day, the ureters are almost constantly in motion. Studies of the lab's engineered ureters have shown that the body acts as a terminal incubator, with cells proliferating *in vivo*. Although in the first few months after a transplant, tissue alignment in specimens is not perfect (some urothelial and muscle cells are not where they should be), there is evidence that architectural reformation is occurring. In specimens taken three months later, all cells are in their proper place. "This shows that if you take normal cells that have all the genetic material present and place them in the right environment," Dr. Atala said, "they will do what they are supposed to do."

The Laboratory for Tissue Engineering and Cellular Therapeutics has also progressed to creating neo-bladders for patients with end stage bladder disease who have no medical recourse. Prior to bladder augmentation surgery, patients come into the hospital for a 3-D CT scan, which creates an image of what their bladder should look like, and a small biopsy of bladder tissue is taken. Committed progenitor stem cells are isolated from the biopsy tissue and are then seeded onto a scaffold and grown *in vitro*. Six weeks later, the engineered bladder is transplanted into the patient and covered with omentum. In a three-month follow-up, patients had significantly decreased bladder pressure from hypertonicity and increased bladder capacities. By six months, compliance was normal, although it declined slightly and temporarily at four months when the scaffold started to degrade and the new tissues took over bladder function.

Other hollow genitourinary tract structures that the laboratory has had recent success in creating include vaginas and uteruses, which have also shown normal morphology. With each organ, the research and development period lessened. “It took us ten years to do what we did to get to the bladder; seven years to develop the vagina; and five years to develop the uterus,” Dr. Atala noted. “We are certainly learning each time we target a new organ.” Recently his laboratory has also constructed blood vessels, nerves, and a trachea, and they are extending their research to solid organs such as the penis, mainly because of Dr. Atala’s interest as a pediatric urologist in children born with congenital problems. Engineered penises were transplanted into rabbits where they functioned relatively normally (80 percent), but more research needs to done to find ways to add more muscle tissue.

Minimally Invasive Cell Therapy

Dr. Atala also discussed the development of minimally invasive therapies for vesicoureteral reflux, a congenital condition that results in the backflow of urine into the kidney and ultimately, kidney damage and loss of the organ. Reflux is usually treated with an open surgical technique in which the ureters are reimplanted in the bladder, a therapy that is 99 percent effective. The minimally invasive technique developed by the Harvard lab involves injecting a bulking agent underneath the ureter, causing a reduction in luminal size, increased resistance, and reduced incidence of reflux. The injectable bulking agent is composed of chondrocytes from the patient’s cartilage mixed with alginate, a substance that has been in existence for many years as a thickening agent for milkshakes. After the bulking agent is injected, it stimulates the growth of other chondrocytes and eventually a soft cartilage forms and the alginate degrades.

The bulking agent was first tested on rabbits with bilateral reflux. The agent was injected below one ureter while the other ureter remained untreated. The treated ureter had reduced reflux and the untreated ureter did not improve. In clinical trials at 10 centers around the country, the biocompatible chondrocyte-alginate mixture improved reflux, as did other bulking agents. However, these minimally invasive therapies were not as effective as reimplantation surgery (66 percent effectiveness versus 99 percent respectively).

The chondrocytes-alginate implantation therapy has also been applied to patients with urinary incontinence. In a Phase I clinical trial of 32 patients, the bulking agent was injected once and had an 80 percent success rate, "which is pretty good for incontinence," Dr. Atala said.

Stem Cells

Most of the cells that the Laboratory for Tissue Engineering and Cellular Therapeutics targets are committed progenitor cells from the tissue or organ that will be regenerated or engineered. This type of stem cell therapy is not immunogenic, which is a problem that would be encountered if embryonic stem cells were allowed to be used.

Therapeutic cloning is another cell therapy that has the advantage of being non-immunogenic. In this type of therapy, scientists would replace the genetic material of a donor egg with the nuclear material from a skin cell that has been removed from a patient in need of therapy. The egg with the new nuclear material would be subjected to a burst of energy that initiates embryogenesis. The genetically matched cells would then proliferate and differentiate and could be injected into the patient.

"Most people associate cloning with reproduction, and they get upset," Dr. Atala said. "But as scientists we must remember, this is not human cloning. This is not the union of a sperm and an egg. It's the union of a skin cell and an egg cell." In addition, some scientists have been concerned that retention of the mother's mitochondrial DNA would cause rejection of the cells.

Dr. Atala's laboratory has studied therapeutic cloning in collaboration with a private company. In one study, they took an egg from a cow and a skin cell from a steer, removed the genetic material of the egg and inserted the nuclear material from the skin cell. The egg after undergoing the blast of energy was then implanted in several of the steer's organs, including the kidney. After a period of time, the implanted cells were removed for analysis, which showed markers for the development of the organs from which they were removed. For example, the implanted cells removed from the kidney had functional and regulatory markers for kidney development.

These developing kidney cells were then seeded onto a reservoir composed of a collagen matrix, and the reservoir was implanted in the back of the same steer. Renal-like tissue formed and produced urine-like fluid. The allogeneic cells were rejected, and the scaffold degraded over time. Histological analysis of the renal-like tissue showed the presence of glomeruli and proximal and distal tubules down to the reservoir, and both regulatory and functional expression of genes and proteins were present. Analysis of the urine-like fluid showed that it was consistent with urine.

"This study showed two things. First, we were able to create tissues using this method. We were able to create cardiac muscle, skeletal muscle, and primitive renal structures

from the skin cell of a steer by using therapeutic cloning techniques. But more importantly, this study was able to show that, in fact, the theoretical concern concerning maternal mitochondria was just that—a theoretical concern. We showed that even with repeated stimulation, these tissues would not reject. Maternal mitochondria does not play a role in rejection of these tissues."

Another way to use stem cells is to target a source outside the tissue source. For example, stem cell populations can be obtained from a patient's bone marrow. These stem cells can be translated and differentiated into many different tissue types. Expanding bone marrow cells is a problem, however. "Bone marrow stem cells are extremely hard to grow and once they coalesce and touch each other, they start to differentiate," Dr. Atala explained. "Special bioreactors would be needed to prevent this from happening, and you would need a room the size of this one to get enough cells for just one patient."

In another study, which has not yet been published, researchers at the Laboratory for Tissue Engineering and Cellular Therapeutics sampled amniotic fluid and placental tissues of 300 pregnant women undergoing amniocentesis and immuno-isolated one specific cell type, which they were able to differentiate into many different tissue types. "These cells grow like weeds, and they do not need feeder layers," Atala said. "We think this system may be a good system to overcome some of the objections to embryonic stem cells. The cells do not produce teratomas when transplanted *in vivo*, and they are multipotential. We think they will be good to study in the future."

The Children's Hospital/Harvard lab has been in existence for 14 years, and 90 scientists have rotated through it in that time. The lab currently employs 25 molecular biologists, cell biologists, chemical engineers, biochemists, and physicians. "Tissues that are studied at our lab are at many different levels of development, and some of them are not ready for prime time," Atala said. "We have the urethra for hypospadias and urethral stricture repair, injectable cells for reflux, injectable cells for incontinence, and bladder tissue is currently in clinical trials." When he and his colleagues believe a technique is ready to be tested on humans, they first perform it on one patient, whom they follow for six months. If everything is functioning well, they try it again on three more patients, following them for three months. Although he was trained as a surgeon, Atala firmly believes that tissue transplantation and cell therapy are the wave of the future.

Questions and Answers

Q: Could you elaborate on some of the organ-specific growth factors or the ability to get some of the cells to grow in culture? Are most of these growth factors and serum-containing media from human sources?
A: No. We actually use defined media. We always start out with basics—serum-free agents. We add growth factors as we provide the media for each particular cell type. Of course that becomes a challenge when you are growing constructs with mixed cell types. Growth factors from one cell type might differentiate another cell type. That's what happens in nature. That's what keeps the systems in check. You want to make sure your

constructs are seeded in the right manner and then you start withdrawing the growth factors that would cause differentiation from one cell type to another.

Q: Are all the scaffolding materials used for various organs from the same background?
A: No, actually they aren't. We take both natural and artificial biomaterials and we have a menu of about 12 different components that we mix and match until we have replicated the properties of the tissue or organ. We're using simple materials, basic building blocks—PGAPLA collagen, etcetera.

Q: How do you handle polymer leaching or any evidence of local inflammation?
A: Very good question. Inflammation is why a lot of the early studies failed. Inflammation is good up to a certain point because inflammation in the scaffold is what's giving you, in a way, the angiogenic supply, but it's a fine balance. You want the scaffold to start degrading but you don't want the inflammatory response to be very aggressive. You do that by slowing down the degradation response. This is done by coating the scaffold. The more you coat the scaffold with your individual cell type, the more protection there is against the body getting to the scaffold.

Q: Can you articulate what taxpayers' dollars do here that's distinct from what private sector dollars do? How should the NIH think about encouraging this research in a way that's not redundant with private sector research?
A: That's a good question. Our lab has no industry funding and, in fact, we shun it. An example is injectable chondrocytes. Injectable chondrocytes was a technology that was developed in our institution and licensed to industry. Industry is not very hot about programs that are going to take many, many years to develop. That's the problem with biologics. Most of the companies now want to concentrate on devices. In fact, most of the major pharmaceutical companies divested themselves of all their biologic companies. The problem is, you need seed funding to get this research to move forward, not only to get it to the level where you can actually apply it in a translational manner.

It's extremely important that you have control of the technology in the development stage and that you're not pressured by industry to accelerate your milestones. That's exactly what happened with the chondrocytes. I basically told the people who licensed the chondrocytes technology that it was not ready for prime time, that it was not ready for patients because the algenate concentration that we were using was good for the laboratory. We were mixing it with the cells and injecting it right then. The company had its own milestones. They needed to get to their milestones to get funding from their own funding agency. Basically, they went through with a product that we did not think was appropriate, and there was nothing we could do about it. They went to Phase I without knowing its efficacy. When it came to the bladder, I was adamant that it would be fully funded by internal sources. In the clinical arena, I don't have to worry about a company telling me I need to meet my milestone. My main concern is for the patient and the patient's well being and to make sure the technology works for other patients as well.