Infectious Diseases and Clinical Xenotransplantation

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[D. Peter Drotman] This podcast series is brought to you by *Emerging Infectious Diseases*, often referred to simply as EID. I'm Dr. D. Peter Drotman, Editor-in-Chief. EID is an open access, high impact, peer reviewed scientific journal published monthly by CDC. EID publishes articles on new and reemerging infectious diseases that occur anywhere around the world so as to improve the understanding of factors involved in disease emergence, control, and prevention.

[Candice Hoffmann] Hi, I'm Candice Hoffmann. On this episode of the *Emerging Infectious Diseases* podcast, we're discussing xenotransplantation.

[Jay Fishman] My name is Jay Fishman. I'm a professor of medicine at Harvard Medical School and director of the Transplantation Infectious Disease Program at Massachusetts General Hospital. And I'm also the associate director of the MGH transplant center. I work at Mass General in Boston where we do both our basic science on xenotransplantation and clinical care of transplant patients.

[Candice Hoffmann] That was Dr. Jay Fishman, lead author of the article, "Infectious Diseases and Clinical Xenotransplantation" in the July 2024 issue of EID.

Organ and tissue transplants can be life-saving procedures. Unfortunately, there is a shortage of organs and tissues. According to the Health Resources Services Administration, 17 people die each day waiting for an organ transplant.

Recent scientific advancements have made xenotransplantation, which is a transplant of animal tissue or organs into a human being, a possible solution to this shortage.

To begin our discussion, let's dive into the definition of xenotransplantation.

[Jay Fishman] Xenotransplantation refers to the implantation of living cells or vascularized organs that are living, between species, and particularly in this case, from non-human species into humans.

When you transplant organs, it comes along with its blood supply, as well as, for example, lymphatics and other connections to the body that are normal. So that what we're trying to do is do organ transplantation so that the organ will live in its normal way, in the normal position in the human body.

[Candice Hoffmann] Although xenotransplantation may sound like a new concept, you might be surprised to learn that people have been experimenting with animal-to-human transplants for centuries.

[Jay Fishman] The history of xenotransplantation is a bit checkered. It goes back to about the 1600s, but there were transplants that are documented from sheep into humans back in the 1600s. There were a series of early corneal transplants from pigs to humans. And then there were some various testicular transplants from chimpanzees into humans who wanted to increase their sexual prowess. And all of that kind of disappeared into history, which is probably a good thing.

And then they tried in the early 1960s to do replacement kidney transplants from chimpanzees and humans. Those didn't work well. We didn't understand the immunology very well. And there was a famous chimpanzee heart transplant into a young child that actually lasted longer than expected back in 1964. Tom Starzl, a famous transplant immunologist and surgeon, did some baboon into human liver transplants in the 1960s. And then there was a long quiescent period when people started trying to do various transplants for islet transplant (pancreatic islets) and the like. So, I became actively involved in the 1980s when we were trying to do pig into primate…non-human primate transplants at that time, looking forward to doing organ replacements in humans, and that point was really studying the immunology and microbiology of xenotransplantation.

[Candice Hoffmann] Sheep, non-human primates, and pigs have been the primary species of animals involved in animal-to-human organ transplant research and experiments. Today, pigs are the most likely source for tissue and organs for successful xenotransplantation.

[Jay Fishman] In general, people have stopped thinking about using non-human primates as organ donors for ethical reasons, but also because of the concern about microbiology and the experience with the AIDS epidemic, knowing that certain primate viruses have the potential for infecting humans. And so, both because people were resistant to using primate organs for human use, which seemed unethical, at the same time there was some microbiologic risk that was associated with that. So, in general, they focused on pigs. And the reason to focus on pigs is that pigs reproduce quickly. They're already being used as part of the food chain. They can be sized appropriately for recipients, and more recently, they can be manipulated genetically to have certain immunologic and physical characteristics that are considered advantageous for transplantation. So, in general, the field has moved towards using pigs as potential donors for clinical use in humans.

[Candice Hoffmann] As scientists work out the risks, benefits, and best practices for infection control, the question now is, how viable is xenotransplantation? That is, how likely is it to be used in healthcare settings around the world?

[Jay Fishman] This is a critical question. The ability to get xenotransplantation routinely into the clinic will depend on clinical trials and some of our early clinical experiences. Our early clinical experiences—and there have really only been four xenotransplants performed in living recipients in the United States and a couple that have been reported out of China—those have been surprisingly encouraging in a number of ways, but that the organs were not rejected, the immune system didn't reject them quickly after transplantation, which is a reflection we can talk about of the genetic manipulations that have been made over the past two decades. But the likelihood is increasing as we learn more about the pig immunology, but also about novel immunosuppressive regimens that have emerged again over the last 20 years that seem to do a better job of preventing graft injury and graft rejection than the protocols we had in the past. We had tried to apply standard immune suppression from the clinic to pig organs and it wasn't sufficient. But with genetic manipulation of the pigs (these are cloned animals), and with better immunosuppressive regimens, we're now able to consider going into routine clinical trials.

And some of the pig into primate experience has been that, for example, pig kidneys can last multiple years in non-human primates. Hearts and lungs and some of the livers have been shorter, and some of those barriers remain. But we've made some big progress, particularly surrounding kidneys and heart transplants. I might also mention that pig transplants into primates

are probably more difficult than pig transplants into humans. There's the size issues, but also primates have a lot of immune responses that we may not see in humans. And so, as a result, these pigs have been genetically engineered to work in humans, but we're putting them into non-human primates. And some of the barriers are greater than what we might have anticipated. So, there's a lot of work left to do to get this into the clinic routinely, but we've made substantial progress over the last couple of years.

[Candice Hoffmann] Some of that progress has been in the field of genetic engineering, which plays a key role in the current research on xenotransplantation.

[Jay Fishman] Most of us will remember the sheep Dolly, which was the first cloned animal, which is a genetically identical animal produced in the laboratory from the transfer of somatic or body cell nuclei into an oocyte that is then put into a host, usually a pregnant or a pseudo-pregnant female, for delivery. And so, that was the original clone idea of cloning, that you took a nucleus from, say, a skin cell, you put it into an oocyte, replacing the nucleus that was there, and you ended up with an animal from a clone.

Now, many years ago, if you wanted to alter the genetics of that clone, you did it randomly. You took genes and you squirted them into the nucleus, and you hope they ended up someplace useful. I did that myself many years ago. It generally didn't work very well, as you might imagine, even though it sometimes did. Gene editing has emerged in the last two decades—and it's also referred to as genetic engineering—where the DNA can be specifically deleted or modified or replaced in that somatic cell nucleus before it's implanted so that the cloned animal then has certain characteristics that are desirable.

What has happened—and you may remember the Nobel Prize was given for CRISPR-Cas9 gene editing—is that you're now allowed to target specific genetic sequences (DNA or RNA sequences) by using a guide sequence that brings it to the right place in the genome. And then the genetic engineering occurs when a double-stranded break is created by an enzyme. And what happens is then the cell itself repairs that break around the inserted DNA. So, what's happening is you're putting it in, you're cutting the DNA, you may be removing a piece, but you can be putting in new pieces. Those new pieces now, we call them cassettes, because that's old terminology for those of you who don't remember, musical cassettes in your car. But the cassettes include multiple genes that are desirable. In the case of xenotransplantation, there were a number of cassettes that were implanted. Some of them were to prevent clotting on the surface of the blood vessels. Some of them were to reduce the immune response to those endothelial or lining layers of the blood vessels. Some of them were to knock out or inactivate viruses that were undesirable in the pig genome. So that you can do a variety of different types of edits at one time, and that's the whole cassette. And so, that's the gene editing in a cloned animal.

Once they're cloned and they reproduce and they're healthy, then you breed them normally, but all of the animals look alike in the sense that they all have the same genetic edits. But you can check, once they're born, are they expressing the various genes that you're looking for in various patterns. So, we've been able to manipulate the genetics of pigs to express or not express up to 62 gene edits. Although, I have to say 59 of them or approximately that, are the porcine endogenous retrovirus, but they have immunologic benefits that now make xenotransplantation a potential clinical reality.

[Candice Hoffmann] With any type of transplantation, infection control is critical. With xenotransplantation, researchers are concerned about possible infections from both pig pathogens and human pathogens.

[Jay Fishman] The big concern in all of transplantation, including human transplantation, is that we're obligated to use immunosuppression to prevent the body from rejecting the graft. And because of that immune suppression, the recipient, whether it be of a human organ or a pig organ, is at risk for infection from the community, from the air, or from the organ, or from other places from their social and sexual partners and the like. So, that we have a sort of formula which says that the risk of infection depends really on two major factors—what you see, the epidemiology of your exposures, and this thing we call the net state of immune suppression, which is how good is your immune system in the presence of immune suppression. So that we know that the longer you're on immune suppression and the more intensive that immune suppression, the greater your risk of infection is going to be.

What are the organisms we're concerned about? Well, there are human organisms that are common, cytomegalovirus, pneumocystis carinii and others, which are common pathogens in immunosuppressed humans. Then you add to that the concern, are the pigs bringing to this equation something new? And even in that, there's sort of different categories. So, pigs have some pretty common bacteria and fungi that are also common in humans. They have some unique bacteria like *Streptococcus suis* that really is only a pig organism. And so, the question comes up, do our laboratories have the capacity to make the appropriate diagnoses should infection occur? The bigger concern is because in transplantation, we target the lymphocytes, particularly the T lymphocytes, to prevent graft rejection, which at the same time predisposes largely to viral infections.

And so, the viruses of pigs have not been completely studied, but I'll tell you the examples that we think about. One of them is the porcine endogenous retrovirus. The porcine endogenous retrovirus would be expected, if it could infect human cells, to cause a greater degree of infection if it was able to infect human cells in an immunosuppressed individual. We don't have evidence for that. The porcine cytomegalovirus is similar to the viruses, the herpes viruses that infect humans. That seems to be a virus that only infects pig cells. However, in our studies, when the pig graft, the xenograft, is infected with porcine cytomegalovirus, you tend to get early graft rejection and you tend to get systemic clotting issues and inflammation. So porcine cytomegalovirus, or PCMV, is undesirable in a transplant.

[Candice Hoffmann] The porcine endogenous retrovirus, or PERV for short, is one of the pathogens discussed extensively in the article.

[Jay Fishman] The porcine endogenous retrovirus is a family of viruses that's present in the genomes, in the normal cells, of virtually every pig that's known. Varying amounts, varying types, but PERV is pretty much a universal characteristic of pigs. My laboratory cloned and sequenced the porcine endogenous retrovirus in about 1997, and a number of other laboratories including a colleague, Clive Patience, was able to demonstrate that the receptors for PERV exist on normal human cells. This made PERV a big concern for potential xenotransplantation because there were concerns that if you put a pig virus into humans, that it would infect human cells. This was a reasonable concern. It turns out that when we looked at people, and this was done, some of the work was done at the Centers for Disease Control, that when people looked at individuals who'd been exposed to pig tissues in the laboratory or in the clinic, people who'd been exposed to

pigs for their lifetime, nobody seemed to have evidence of PERV infection. So, it seemed not to be occurring, but it was a concern. In the laboratory, it was shown that PERV was able to infect only virally changed cell types. So, normal cells didn't seem to be easily infectable by PERV, lending another sense of safety to this idea that PERV might not infect normal human cells. But it was a concern. So, that some of the companies that are producing pigs for xenotransplantation have now developed pigs that have inactivated PERV genes. They've actually gone in with CRISPR-Cas9 and inactivated the polymerase gene of these endogenous retroviruses.

So, we don't know what the actual level of concern should be. And many pigs have different characteristics. They lack certain PERV genes. They have other PERV genes. But in general, there are three families of PERV called A, B, and C. And it turns out that C recombines with A to make a more virulent form in the laboratory. So that some of the individuals who are using pigs are using PERV-C-negative pigs. Again, we don't know whether there's any risk or not, but we think that that risk, if it exists, is quite small based on laboratory experience.

[Candice Hoffmann] New or previously undiscovered pathogens could be a concern with xenotransplantation. However, the emerging field of metagenomics could help with that.

[Jay Fishman] Well, there's a whole field now in genetic sequencing called metagenomics. And what we're capable of doing is sequencing everything, DNA and RNA, that's circulating in an individual's blood. And then we compare it to a database of all the known sequences of infectious organisms that exist. And so, using metagenomics, we can look for the unknowns, both organisms that have been seen before and organisms that might look like something we've seen before. And so, this is a very exciting area, not just for xenotransplantation, where we can screen people and monitor them for potential infectious exposures that we might miss otherwise, but also to apply to all of infectious disease to think about looking for organisms in people where we've been unable to make a clinical diagnosis. So, one of the benefits of using metagenomics in xenotransplantation may be broader in the whole field of transplantation and in infectious disease.

[Candice Hoffmann] Broadening the available laboratory tests for pre-transplantation screening is a growing area of research.

[Jay Fishman] There are very good microbiology laboratories associated with virtually any clinical center that does organ transplantation. We see all kinds of infections in all of our recipients, and we've come to expect them, and we learn how to treat them. What if you don't have the right assay? So, these assays, many of them are highly specific. They're what we call nucleic acid amplification tests (NAATs) or polymerase chain reaction assays. Those are specific and they amplify a specific sequence so that we can detect it within our assays. What if you don't have the right genetic sequence? What if you're looking for the unknowns? Well, as I mentioned, the metagenomics or the possibility of sequencing everything in the circulation or in an organ biopsy allows us the opportunity to look for things that we might not expect. And so, what we're combining is standard microbiology, a certain amount of veterinary microbiology because there's very good veterinary labs that work in xenotransplantation, and then genetic sequencing or metagenomics to start looking for what we don't know about. So, there's a tremendous opportunity to broaden microbiologic testing and we have to, to take good care of xenotransplant recipients.

[Candice Hoffmann] Screening tests before transplantation help prevent infections. However, infections still happen.

[Jay Fishman] In human allotransplantation (human-to-human transplantation), we do a certain amount of screening (microbiologic screening) before we accept an organ for transplantation. Most of those are based on antibody or serologic testing. Some of them are nucleic acid tests. And we get those data before the organ is implanted in general. Conversely, many of the microbiologic tests we do on donors are not available until the organ has already been implanted. And so, although the rate of donor-derived infection is quite low, less than 0.1%, it does happen. And that's because, in part, our screening is not complete, but also because it's not timely. We don't have the time to wait to find the results of our testing before the organ has to be put into the human recipient. Can't keep the organ alive outside the body for that long. Technology is improving, but we're still not there yet.

[Candice Hoffmann] This is where xenotransplantation might have a leg up on allotransplantation. There are infection control steps unique to xenotransplantation that might help prevent infections.

[Jay Fishman] So, one of the fundamentals of xenotransplantation is that these animals, both normal animals and cloned animals, are bred in isolation in bio-secure facilities so that they can't be exposed to other pigs, to birds, amphibians, humans, and other sources of infection. And then they're tested extensively, both for pig organisms and human organisms, and non-specifically, to assure that they don't have infections that could potentially be infectious for humans. But once we've taken those animals and tested them, we keep them in this bio-secure facility until they're used. They're transferred in a bio-secure van to the clinical place. So that what we're trying to do is assure that there are not organisms in the pigs that can be transferred to human recipients. And similarly, guaranteeing that they're healthy, socialized, have contacts with other animals so that they're well taken care of in this facility. But they don't pose a risk of transmission of infection to the human, immunosuppressed human recipient. So, we monitor the pigs, we monitor the human recipients, and in fact, we store blood samples on our staff in case some infectious episode should occur. So, there are occupational health considerations as well.

[Candice Hoffmann] So, when can we expect to see xenotransplantation in wide use?

[Jay Fishman] My crystal ball is probably a little different than the crystal ball at the Food and Drug Administration. They have been very supportive, I should say. But they have put on certain regulations, and there exist certain regulations, for the performance of clinical trials, prospective clinical trials. The clinical recipients that have received pig organs to date have been compassionate use individuals who had no other options other than a pig organ. But in the future, where we'll be doing routine informed consent and education, education of the staff of the hospital and others, we will have to abide by FDA regulations in terms of getting those into clinical trials. And so, there are some guidelines for those clinical trials, but our recent experience will inform those guidelines as we move forward. I should point out that this research is not only occurring in the United States, but in Europe and Australia and in China and elsewhere. So that work is ongoing in parallel in a number of places. And there is an extraordinary degree of collaboration amongst various groups, particularly within the United States, Europe and Australia, to try to move this field forward safely but quickly.

[Candice Hoffmann] Moving this research forward will require collaboration across many disciplines.

[Jay Fishman] Different groups have different preclinical and clinical experiences. So, most of the groups who have been working on xenotransplantation have active human transplantation

programs. But you need a number of things. You need technical skill. You need immunologic skill. You need microbiologic skill and experience. But you also need pigs. And so, what has happened is that the scientific community has been very quick to publish their results and there is a series of commercial companies that have started producing pigs and that are taking responsibility not just for producing the pigs for organ transplantation but doing microbiologic screening, creating bio-secure facilities. And so that each center has collaborations with one or more companies that are producing these pigs, multiple research laboratories, and in some cases commercial research laboratories for doing genetic sequencing, for example, and between the clinical centers. And so, the major clinical centers, places like Massachusetts General Hospital, NYU in New York, the Langone Center, University of Alabama, and Birmingham, places that have done…University of Maryland where they did two heart transplants, we all know each other, we all talk to each other routinely, and we share experiences. And part of it is that there are a fairly small number of places doing clinical transplants or preparing to do clinical transplants. And part of it is transplantation in general is a reasonably small field. And so that anything that can inform the field and make it safer and move the field along more quickly is an advantage to all of us.

[Candice Hoffmann] Dr. Fishman has this advice for future researchers who want to know more about xenotransplantation.

[Jay Fishman] The field of xenotransplantation is fairly broad. It has informed us in terms of developing immunologic techniques for studying donor and recipient matches, microbiologic techniques for looking for known and unknown organisms, and novel immunosuppressive therapies. So, there is an extraordinary breadth of opportunity in this field in terms of basic and clinical research. And the advantage of xenotransplantation is that there's something there for everybody. If you want to study ethics, if you want to study pig or animal rights, if you want to study psychology or psychiatry related to a new field, all of these have been started as a result of xenotransplantation research. I think we have informed occupational health protocols, we have informed pig breeding, and certainly veterinary practice has benefited from studies in xenotransplantation. As I've mentioned, we're developing validated microbiologic assays as part of xenotransplantation. So, if you want to get into a field that's exciting, that has clinical applications like organ transplantation, you're saving lives. And that's the goal. The goal is to save lives and to be safe while doing it. Are there risks? Yeah, there are some risks. But transplantation has always been associated with some risks. It's always been cutting edge research. But it is an opportunity to get into an exciting community. And I talked previously about collaborations. We have collaborations. We talk to each other, both within our institutions and across institutions. So, if you want to find an area, you can come join societies that are discussing xenotransplantation. So, there's an International Xenotransplantation Association. There is the American Society of Transplantation. All of us have interest in xenotransplantation. And it is an opportunity to get into a community where there's a lot of very interesting research going on.

[Candice Hoffmann] So, what's the bottom line?

[Jay Fishman] Xenotransplantation for clinical application is likely to occur in the next three to five years. We are doing everything we can to make this as safe and effective as possible through studies of immunology and microbiology and immunosuppression. It is a tremendous opportunity for our patients and for the community and also in terms of the knowledge to be gained in the field of transplantation. So, the simplest summation is that xenotransplantation is

very exciting, but it's actually starting to occur. And so, we're looking forward to a lot of rapid progress in the next three to five to 10 years.

[Candice Hoffmann] If you've enjoyed this podcast, we encourage you to check out *Emerging Infectious Diseases* online. Dr. Fishman explains why he's a regular reader.

[Jay Fishman] So, *Emerging Infectious Diseases* captures a lot of epidemiology that's not present elsewhere in the literature. I am an infectious disease specialist. I read the infectious disease journals. I read the basic science journals and the transplantation journals. But EID has a unique set of both case reports and reviews looking at epidemiology so that the reflection of the work that's being done in epidemiology in the laboratory as well as the CDC, I think is great to capture in one place. It's available online and I read it routinely.

[Candice Hoffmann] Thanks for listening to our podcast. You can read the *Emerging Infectious Diseases* journal at cdc.gov/eid. You can also follow EID on X and Instagram @eidjournal, and on LinkedIn @eid-journal.

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