Discordant cardiac xenotransplantation: broadening the horizons

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INTRODUCTION—WHY DISCORDANT PIG CELLS AND ORGANS?

The various Eurotransplant waiting lists include almost 15 500 patients, 1451 of which died in 2011. The situation is particularly poor for people needing heart transplantation, with 226 of 1277 (17.7%) candidates in that category dying in 2011 [1]. Several alternatives have been suggested to overcome the grave shortage of organs. One possible solution would be clinical xenotransplantation using non-human primates as concordant donors and triple drug immunosuppression as applied using human allotransplants [2]. However, ethical and logistical considerations preclude this. Apes are endangered species and their use is out of the question, and other non-human primates are too small and their growth too slow. In contrast, discordant species, notably pigs, offer an abundant new source of organs and cells. Their gestation time is short (~4 months), pigs have multiple offspring (10–12 per litter) and mature in 6–8 months. Pigs have been bred for food for centuries in many parts of the world, so ethical objections should be minor. The main disadvantages arise from disparities between swine and primates resulting from >90 million years’ evolutionary divergence, which can affect important protein–protein and other biochemical interactions.

IS THE SHUMWAY PARADIGM STILL VALID?

The great Norman Shumway used to say, ‘Xenotransplantation is the future, and always will be!’ Fortunately, this pessimistic view is no longer true. In a recent review, Ekser et al. [3] listed the longest survival times reported for xenografted porcine cells and organs. Microencapsulated pancreatic islets (Fig. 1) from wild-type animals survived >800 days [4]. Non-encapsulated islets transgenic for the human complement regulatory protein CD46 survived almost 400 days [5]. Abdominally placed heterotopic (non-working) double genetically modified (CD46 and α-Gal-KO) hearts beat for up to 236 days [6], while orthotopically placed organs were life supporting up to 57 days [7]. Transplanted CTLA4 transgenic neuronal cells to treat Parkinson’s disease, and wild-type decellularized corneas were also successful for hundreds of days [8, 9]. Whole kidneys from CD55 transgenic animals survived for 90 days [10], although porcine renal erythropoietin is not recognized by primate recipients and must be replaced. Liver and lung transplants do, however, stand in contrast, surviving only for 8 and 5 days [11, 12].

There are a dozen groups (seven in the USA, two each in Australia/New Zealand respectively Asia, one further in Europe) around the world pursuing xenotransplantation research. Our experience in this area started in Munich in 1998 with funding by the Bavarian Research Foundation; in 2004 the German Research Foundation took over support and the Hannover groups joined; 2012 marked the beginning of the Collaborative Research Centre and inclusion of the Dresden diabetes group. The strength of our Consortium is the mix of basic researchers, animal biotechnologists, virologists and clinicians. Our main goal is to achieve clinical experience with genetically modified porcine islets and decellularized heart valves (from α-Gal- and Hanganutziu–Deicher antigen knock out animals), kidneys and hearts.

Testing the efficacy and safety of genetically modified (gm) cells, tissues, organs and other products is an iterative process. This commences with in vitro testing of efficacy using appropriate pre-existing and purpose developed biochemical and biological laboratory procedures. Small animal experiments, valvular stability tests or solid organ perfusion follow. Work then proceeds to preclinical non-human primate studies. As issues are identified, the cycle starts anew. In this way, we aim to generate clinically safe gm porcine products free of any infectious risk (for further information of our Consortium see http://www.klinikum.uni-muenchen.de/SFB-TRR-127/de/index.html and http://www.dfg.de/foerderung/programme/listen/projektdetails/index.jsp?id=213602983).
Porcine cellular and organ xenotransplantation in non-human primates has so far been technically demanding, time consuming and costly, but the rewards for human health are considerable. Transplant experiments necessarily depend on the availability of gm donor animals, which up to now have mostly been generated directly by nuclear transfer cloning, a laborious undertaking that can have an uncertain outcome. This is, however, set to change in the near future. Our Consortium has access to lines of gm pigs (Table 1) of both sexes, enabling production of donor animals carrying multiple genetic modifications by conventional breeding, allowing transplant procedures to be planned on a regular basis.

**PORCINE PANCREAS ISLETS WILL BE FIRST IN THE CLINIC**

Porcine insulin is itself recognized and functional in primate recipients; however, without immunosuppression, unmodified porcine insulin producing cells succumb to early graft loss known as ‘immediate blood-mediated inflammatory reaction’ driven by preformed antibodies, complement and excessive coagulation. Encapsulation obviates the need for immunosuppressive drugs. Islets are surrounded with a porous biopolymer, composed mainly of alginate (Fig. 1). The pores are large enough for small molecules like water, glucose, oxygen and most importantly insulin to permeate, but exclude cells and larger molecules such as antibodies. However, it is not known how long these capsules can maintain their function in vivo, since loss of integrity or occlusion of the pores would be problematic.

The New Zealand company Living Cell Technologies is a pioneer in the field, having so far treated 22 diabetic patients suffering from frequent episodes of unaware hypoglycaemia (http://www.clinicaltrials.gov/; trial number: NCT 00940173 and www.lcctglobal.com). A dose finding and safety study showed improvement in some treated patients, for example reduced glycated haemoglobin levels. Most importantly, however, there was no evidence of zoonoses and no sign of activation of porcine endogenous retroviruses, a theoretical risk that has long been recognized [15]. The genetically unmodified donor animals were from a designated pathogen free (DPF) herd that originated from a feral breed mainly of alginate [14] (Fig. 1). The pores are large enough for small molecules like water, glucose, oxygen and most importantly insulin to permeate, but exclude cells and larger molecules such as antibodies. However, it is not known how long these capsules can maintain their function in vivo, since loss of integrity or occlusion of the pores would be problematic.

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DISCORDANT XENOGENEIC HEART TRANSPLANTATION

Opening of the aortic clamp allows primate (baboon) blood containing preformed antibodies to perfuse the porcine coronary arteries where they bind pig antigens, mainly α-Gal sugar epitopes (see glossary, α-Gal). Within fractions of a second, various complement reactions are activated leading to formation of the membrane attack complex (MAC) and destruction of graft vasculature (Fig. 2). Subsequent interstitial haemorrhage and oedema lead to graft failure, which is well documented as the hyper-acute rejection reaction.

If this can be circumvented, the next obstacle is presented by new non-α-Gal antibodies formed within ~3 weeks of grafting, starting the delayed humoral response. Similar to a time span, the effects of protein incompatible within the coagulation system also become evident. For example, porcine thrombomodulin (TM) binds only weakly to primate thrombin, leading to levels of activated Protein C insufficient to interrupt coagulation effects, resulting in occlusive thrombotic microangiopathy in transplanted porcine organs within weeks postoperatively.

Over the longer term, cellular rejection signs are similar to those observed after allogeneic procedures.

Multiple gm pigs are central to our strategies to minimize or even abolish hyper-acute and delayed humoral rejection reactions. Modifications include removal of α-Gal epitopes by genetic knock out; hyper-expression of the complement regulators CD46, CD55 and CD59 to block complement reactions initiated by secondary induced antibodies and expression of human TM to overcome the cross-specific protein incompatibility mentioned earlier, ensuring Protein C activation and anticoagulation (Fig. 2). Table 1 provides a more complete list of the genetically modified pigs available to our Consortium. Up to now, we have used double (α-Gal-KO and CD46) and triple (α-Gal-KO, CD46 and h-TM) gm pigs in our experiments, and most recently quadruple (α-Gal-KO, CD46, h-TM and LEA29Y) gm pigs are available. Animals that also express hemoglobin-1 and HLA-E are currently being generated and will be available in the near future.

Xenogeneic heart transplantation will clearly need additional immunosuppressive strategies. Since the time of the procedure is known in advance, the bone marrow (and therefore antibody production) is suppressed before transplantation using anti-CD20 to destroy B-cells, bortezomib in combination with cortisone to destroy plasma cells, and cyclophosphamide [16]. Extracorporeal immunoadsorption is used to remove pre-existing α-Gal and non-α-Gal antibodies and any antibodies formed postoperatively. Maintenance immunosuppression is provided by tacrolimus, mycophenolate and cortisone as for allogeneic transplants; antithymocyte globulin induction therapy is also applied.

We have so far achieved 50 days’ survival in the unique thoracic heterotopic (working) heart transplantation model developed by Barnard and Losman [17, 18] using this immunosuppressive treatment (Fig. 3A and B).

Further improvements will require additional immunosuppressive treatment to overcome the delayed humoral rejection reaction, which has been difficult to treat. Total thoracic lymph node irradiation is an additional procedure and can be combined with costimulation blockade of the CD40/CD40L system with antibodies ([6], Fig. 2). CTLA4 (or the more potent LEA29Y) can be used to turn off another costimulating system (that of CD28 and CD80/86). Conveniently, LEA29Y may be expressed as a transgene by the donor pig (Table 1). Transgenic pigs that express LEA29Y specifically in the pancreas are available and have been used successfully in diabetic humanized immunodeficient mice by one of the projects within our Consortium [19]. Together with other modifications, cardiac specific LEA29Y is also available now.

Our main aim is to fulfill the guidelines of the Xenotransplantation ISHLT Advisory Board [20] and achieve good graft function for a minimum of 3 months in a life-supporting position in at least 60%
of our consecutive experiments. This has not been achieved so far, since it is difficult to meet within the laboratory setting. Future results of cardiac xenotransplantation must be compared with recent 6-month 60% patient survival after implantation of biventri-
cular continuous-flow mechanical assist devices—and of course the near 90% survival of those patients without right ventricle dis-
function, in whom simple left ventricular assists work well [21].

Despite these improvements, however, mechanical cardiac support systems carry permanent substantial long-term risks. These include cerebrovascular accidents, caused not only by adverse bleeding effects but also by thrombus formation within the mechanical tools, and gastrointestinal bleeding as a result of an acquired device-related von Willebrand factor deficiency [22]. Percutaneous drivelines also represent a risk of infection.

Initial clinic xenogeneic heart transplantations may, therefore, provide a solution for heart failure patients with severe right ventricular disfunction, coagulation disorders or difficult anatomical preconditions such as small left ventricles or significant aortic incompetence. A high antibody titre against the HLA system will be an early indication since the SLA system does not crossreact ([23]; reviewed in ref. [24]).

More recent WHO meetings have been held to discuss regu-

Numerous prerequisites were summarized and will be addressed within the rules and regulations of the European Medicines Agency, which in Germany is represented by the Paul-Ehrlich-Institute.
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Conflict of interest: David Ayares is employee of Revivicor (Blacksburg, VA, USA), and Robert Elliott and Paul Tan are employees of Living Cell Technologies (Auckland, New Zealand).

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[14] Elliott RB, Escobar L, Tan PL, Garkavenko O, Cala A et al. Three-dimensional reconstruction of a computer tomography for continuous monitoring of aortic pressure is indicated as S (computer tomography by courtesy of Fabian Bamberg, Department of Radiology, LMU, Munich, Germany). (B) ECG of the donor graft (g) and blood pressure curve of donor and recipient (r) hearts in the ascending aorta after cardiac heterotopic thoracic xenotransplantation (postoperative day 10).

Figure 3. (A) Three-dimensional reconstruction of a computer tomography after cardiac heterotopic thoracic xenotransplantation from pig to baboon. The native heart (right side) and the porcine heart (left side) are anastomosed between both atria (not visible). The two main pulmonary arteries were joined end-to-side by interposition of a graft (P). The aorta of the porcine heart and the native aorta are anastomosed directly end-to-side (A). The pressure probe for continuous monitoring of aortic pressure is indicated as S (computer tomography by courtesy of Fabian Bamberg, Department of Radiology, LMU, Munich, Germany). (B) ECG of the donor graft (g) and blood pressure curve of donor and recipient (r) hearts in the ascending aorta after cardiac heterotopic thoracic xenotransplantation (postoperative day 10).