

Role of Intrinsic Factors in the Growth of Transplanted Organs Following Transplantation

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Abstract

Shortages in the availability of transplantable organs have forced the transplant community to seek alternative methods to increase the supply of available organs. In our recent study following α -1,3-galactosyltransferase knockout (GalT-KO) pig-to-baboon kidney xenotransplantation, we found that certain recipients developed increased serum creatinine, possibly due to the rapid growth of orthotopic pig grafts in smaller baboon recipients. To test our hypothesis, we assessed whether the growth of outbred (Yorkshire) organ transplants (kidney and lung) in miniature swine was regulated by intrinsic (graft) factors. Yorkshire kidneys reached 3.7x their initial volume over 3 months vs. 1.2x for miniature swine kidneys over a similar time period. A similar pattern was seen in porcine lung allografts as well. Following xenotransplantation, a review of our results suggests that there is a threshold for kidney graft volume of 25 cm³/kg of recipient body weight at which cortical ischemia is induced in transplanted GalT-KO kidneys in baboons. These results suggest that intrinsic factors are in part responsible for the growth of donor organs and this should be taken into consideration for growth-curve-mismatched transplants.

Short Communication

We recently published data in the American Journal of Transplantation looking at “The role of intrinsic (graft) vs. extrinsic (host) factors in the growth of transplanted organs following allogeneic and xenogeneic transplantation” in an effort to better understand what factors contribute to organ growth and development following transplantation [1]. Organ development and growth involves a complex cascade of signalling molecules and pathways, which are essential following transplantation and recent work by Andersen et al., in a *Drosophila* model, analysed key hormones and pathways responsible for organ development [2]. In an effort to better understand growth and development in large animal transplant models, this short communication will introduce you to our recent findings observed following renal and lung allo and xenotransplantation.

With improvements in medical therapy the incidence of end-stage organ disease continues to increase, as does the need for transplantable organs, creating a vast discrepancy between the number of patients on waiting lists and the number of available donor organs. Given this shortage, xenotransplantation offers the potential benefit of an inexhaustible supply of organs with swine being the likely ideal donors given their physiologic and anatomic similarities to humans [3]. Despite these similarities, in order for xenotransplantation to become successful several key factors must be addressed. Due to because of varying litter sizes and susceptibility to genetic modification, most research institutes [4,5], except a group in Kagoshima, Japan [6] and ours [7] use Yorkshire or Landrace swine, which can undergo genetic modification. These domestic wild type swine reach body weights of over 100 kg by 6 months [8] and grow to approximately 400 kg. Therefore, growth rates of xenografts, especially for “life supporting solid organs” remains an important issue to be

addressed. As interest in xenotransplantation grows, recent improvements in genetic engineering using the CRISPR/CAS9 technology have substantially increased the efficiency of gene modification and the use of these new multi-transgenic swine have led to marked prolongation of porcine xenograft survival, for example, the recently reported survival of a CD46/hTBM GalT-KO heterotopic pig-to-baboon heart transplant recipient for over 2 years [9].

In an effort to bring xenotransplantation into clinical reality, our lab has long been testing a strategy to induce tolerance through the co-transplantation of vascularized porcine thymus in a pig-to-baboon large animal model. Our initial study has demonstrated that a porcine kidney that is co-transplanted with a vascularized porcine thymus from the same donor can achieve survival for up to 83-days in baboons using galactosyl- α -1-3-galactosyl transferase gene knockout (GalT-KO) pigs as donors [10]. More recently, we achieved greater than 6-months survival of a baboon recipient bearing a life-supporting GalT-KO kidney with vascularized thymus, and have increased the average survival to >125 days that following a modified immunosuppressive regimen [10]. However, retrospective analysis revealed that certain recipients maintained a normal serum creatinine for the first 2-3 months post-transplant, but then gradually experienced an increase in Cr without evidence of rejection (determined by absence of elicited anti-pig antibodies or T-cell sensitization). Further histologic examination of these grafts at the time of euthanasia demonstrated cortical ischemia without vasculitis thus leading us to hypothesize that the rapid growth of the porcine xenograft in these small baboon recipients may have led to deterioration of organ function due to a form of abdominal compartment syndrome causing diminished intra-graft blood flow leading to cortical ischemia. These observations led us to hypothesize that the rapid growth of these orthotopic pig grafts might have led to deterioration of organ function due to a form of

compartment syndrome within the abdomen of the baboon, causing insufficient intra-graft blood flow.

To further test this hypothesis, we then examined: 1) the apparent threshold volume that induces cortical ischemia in transplanted life-supporting GalT-KO kidneys in baboons; and 2) the growth of outbred, Yorkshire swine-to-miniature swine kidney and lung transplants to determine whether growth of these organs is regulated by intrinsic (graft) factors [1]. Previous studies have demonstrated that pig organs grow at a much faster rate those of primates [11], with Yorkshire swine reaching their maximum body weight of over 100 kg by 6 months [12], while miniature swine reach their maximum weight of only 40 kg at 6 months [3,13]. This discrepancy in growth allowed us to test our hypothesis by examining the threshold volume at which point cortical ischemia would occur following life-supporting GalT-KO pig-to-baboon kidney xenotransplantation, as well as following allotransplantation of outbred Yorkshire swine-to-miniature swine kidneys and lungs to determine whether growth was regulated by intrinsic (graft) or extrinsic (host environment) factors.

For our allotransplantation model, five miniature swine received allogeneic kidneys; three were from size-matched Yorkshire pig donors (experimental) and two were from size-matched miniature swine (control) at the day of transplantation. Additionally, three miniature swine received left orthotopic size-matched lungs from Yorkshire pigs. The transplantation of Yorkshire lungs into miniature swine was done to demonstrate the effects of transplanting organs from the donors that naturally grow faster than recipients into a small, limited thoracic cavity. At the time of xenotransplantation both the donor and recipient lungs were the same size thus allowing us to determine whether intrinsic (graft) factors promote, at least predominantly, organ growth since extrinsic (growth) factors should be compatible between swine allotransplantation. For the xenotransplantation model, five baboons received a miniature swine GalT-KO xenokidney, with two recipients treated with an anti-CD154 plus Rituximab based regimen and the other three receiving a modified regimen that included CTLA4-Ig and MMF converted to intra-muscular (IM) rapamycin beginning three weeks post-transplant. Kidney and lung graft biopsy specimens were examined and graded by an experienced pathologist. Biweekly renal ultrasound examinations were performed to monitor allograft volume changes. Kidney volumes were determined from the maximum measured length, width and depth, using the formula: $(\text{length} \times \text{width} \times \text{depth} \times \pi/6)$ - the volume for an ellipsoid [14]. Lung allografts were assessed by physical examination and by chest X-ray. Following euthanasia, grafts were explanted, measured and weighed.

Our results seem to suggest that following transplantation in both allogeneic and xenogeneic models intrinsic factors within the transplanted organ are largely involved in graft growth. In the xenogeneic model, baboon recipients of GalT-KO thymokidneys without additional gene modification obtained prolonged graft survival for up to 193-days, however, when the ratio of the donor pig kidney volume to the recipient body weight was $>25 \text{ cm}^3/\text{kg}$ there appeared to be a deleterious effect on renal function, despite xenogeneic thymokidney recipients demonstrating long-term survival and anti-pig unresponsiveness *in vitro*, as well as anti-pig cellular and antibody assessments and evidence of new thymic emigrants from cotransplanted thymic grafts of thymokidneys in the recipients, likely as a result of baboons not being able to grow as fast as pigs. The growth index, which takes growth curves of hosts into account (kidney volume/recipient body weight), appears to be a more appropriate assessment of non-immunologic graft dysfunction due to size

mismatch than a simple measurement of width, depth and height of the xenografts or percentage increase in volume of the xenografts.

In the allogeneic kidney model, porcine kidney grafts were transplanted in an orthotopic (intra-abdominal) fashion, allowing the kidneys to maintain stable renal function over the course of 280-days and grow without compression until the graft reached the right lobe of the liver or the laterally located pelvic bones. After this period, the percentage increase of kidney volume/body weight from outbred Yorkshire swine transplanted into miniature swine increased 3x more than those of grafts from miniature swine transplanted into miniature swine at 12 weeks post-transplant. In the allogeneic lung model, the rapid growth of Yorkshire lungs caused compression, presumably due to the fixed rigid thorax, and this led subsequently led to the development of atelectasis and pneumonia in the upper lobes of the allografts.

The observed results indicate that xenogeneic kidney grafts demonstrated decreased graft function, due to insufficient cortical blood flow due to continuous growth of pig kidneys in the limited baboon abdominal cavity. Similar to xenokidneys, Yorkshire lung grafts experienced deterioration in graft function with prominent atelectasis in the upper lobe due to lack of accommodation for growth in the mediastinum, where the thoracic cavity is rigid and does not allow for further expansion.

Interestingly, cellular factors, including molecular and genetic pathways involving growth hormones (e.g. the insulin receptor-phosphoinositide 3 kinase (PI3K) or telomerase pathways), as well as environmental factors, such as hypoxia, have been known to be involved in organ growth [15,16]. Growth hormone most importantly may be a critical factor for organ growth following transplantation. Behncken et al. have reported that porcine growth hormone did not activate human hormone receptor due to amino acid differences of the growth hormone structure between species [17]. In contrast, previous studies have demonstrated that human growth hormone could still have biological effects in pigs [18]. Considering these conflicting results, further studies should be performed to determine whether extrinsic factors regular growth of transplanted organs. However, it would seem to indicate, by our results, that rapid growth of Yorkshire kidneys and lungs in miniature swine as well as growth of pig kidneys in baboons indicate that factors intrinsic to the organ may play an important role in transplanted grafts.

As with kidneys and lungs, Brenner et al. similarly experienced considerable increases in organ growth following cardiac xenotransplantation where size mismatched donor hearts filled most of the right thoracic cavity and caused compression of the surrounding lung parenchyma [19]. Differences in methods for cardiac xenotransplantation, i.e. heterotopic, orthotopic and intra-thoracic heterotopic, have all been well described [20], however, despite heterotopic transplantation having the benefit of unlimited growth in the abdomen as opposed to orthotopic heart transplantation into the mediastinum, the heterotopic abdominal approach is not life-supporting and true orthotopic models have been complicated by an entity known as perioperative cardiac xenograft dysfunction (PCXD) requiring additional investigation, currently ongoing.

In summary, our results, from transplantation studies indicate that above $25 \text{ cm}^3/\text{kg}$ (kidney volume/recipient body weight) cortical ischemia appears to occur following pig-to-baboon kidney xenotransplantation. Furthermore, organ growth appears to be predominantly regulated by intrinsic factors and deterioration of organ

function is observed earlier for orthotopic allo-lung transplants compared to allo-kidneys, presumably due to the narrow mediastinum when compared to the abdomen. These results suggest that intrinsic factors are responsible, at least in part, for growth of donor organs and as a result additional approaches to gene manipulation in order to control growth of swine would be able to limit growth of swine organs for clinical xenotransplantation and this unique characteristic should be taken into consideration for growth-curve mismatched transplants, for example pediatric patients.

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