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Toward a solution for cardiac failure in the newborn

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The newborn infant with severe cardiac failure owed to congenital structural heart disease or cardiomyopathy poses a daunting therapeutic challenge. The ideal solution for both might be cardiac transplantation if availability of hearts was not limiting and if tolerance could be induced, obviating toxicity of immunosuppressive therapy. If one could safely and effectively exploit neonatal tolerance for successful xenotransplantation of the heart, the challenge of severe cardiac failure in the newborn infant might be met. We discuss the need, the potential for applying neonatal tolerance in the setting of xenotransplantation and the possibility that other approaches to this problem might emerge.

Key words: Xenotransplantation; Hypoplastic left heart syndrome; Neonatal tolerance; Organ replacement

Xenotransplantation has been approaching clinical application for many years. What might seem a leisurely approach to clinical application belies rapid and gratifying progress in identifying and overcoming biological barriers **(1)**. The rapid progress in xenotransplantation however has been equaled and sometimes exceeded by advancing standards for entry into clinical practice (a process called 'moving the goalposts'). Viewed from a distance, then, xenotransplantation appears to be engaged in a marathon with alternative medical and surgical treatments, including allotransplantation, all potentially slowed by regulatory hurdles. The course of this marathon is eloquently brought into focus by David Cooper and colleagues **(2)**, who discuss the prospects for applying neonatal tolerance to advance xenotransplantation as a treatment for congenital structural heart disease and cardiomyopathies.

Cooper et al. **(2)** assert, no doubt correctly, that porcine hearts (and presumably other organs and tissues) would be acceptable alternatives to scarce human hearts as sources of transplants if tolerance to the source of the transplants could be safely and effectively achieved. Implicit in the proposition is an assumption, we think warranted, that concerns about transmission and/or generation of novel infectious agents have been overblown. The authors base their proposition on the observation that tolerance to blood group antigens A or B of the donor occurs in the preponderance ABO-incompatible cardiac transplants in the newborn (e.g. based on negative ELISPOT for blood group specific B cells, tolerance developed spontaneously in each of 13 newborn recipients of ABO-incompatible cardiac transplants **(3)**). The proposition could draw further support from observations that a small fraction (perhaps 5%) of mature recipients of kidney or liver allografts develop "operational tolerance" to donor HLA **(4)**, i.e. the grafts in mature recipients survive and continue to function despite discontinuation of immunosuppression. Further, as the authors note, tolerance to has been deliberately induced in small numbers of mature kidney allograft recipients **(5, 6)**. Cooper and colleagues would thus employ a hybrid approach - both the spontaneous tolerance of the newborn and deliberate induction of tolerance - to support the transplantation of porcine hearts in newborn afflicted with severe cardiac failure or cardiac malformations not amenable to surgical palliation.

The proposal takes as one assumption that spontaneous tolerance and induced tolerance are not mutually inimical. We accept that premise for the present, although we have observed non-pathogenic autoimmunity in subjects who underwent cardiac transplantation in infancy **(7)**. The proposal seems to take as a related assumption that a newborn (or a fetus) is more accepting of foreign antigens and potentially of transplants than an adult and potentially more amenable to induction of tolerance. That belief is based on more than a century of observation **(8, 9)** (although not beyond dispute **(10)**). The authors believe these assumptions together with the dramatic progress in extending the survival of xenografts beyond a year **(11-13)** make newborn patients with severe cardiac failure ideal subjects in whom to test induction and sustained maintenance of xenogeneic tolerance and therefore ideal recipients for cardiac xenografts. After all, the authors point out, xenogeneic tolerance would spare the recipient (and the insurance company or government) a lifetime of immunosuppressive therapy and if the xenograft failed (as do some allografts) the recipient might be rescued by a human allograft. We certainly agree that safe and effective induction of xenogeneic tolerance would have many benefits for the recipient, but we are skeptical about whether and how easily tolerance to antigens other than saccharides can be achieved, even in the newborn.

Implicit in Cooper's proposal is one further assumption - that staged reconstruction of the hypoplastic left heart and other 'single ventricle' malformations, as effective as it might be, is not the ideal solution. We agree. Nearly half of those who survive the Fontan procedure nonetheless die or require transplantation within six years **(14, 15)** and transplantation after failed reconstruction has a greater risk of failure than a primary transplant **(16)**. We think that cardiac transplantation, when available, provides a better therapeutic option and the only possibility of normal physiology. But, allogeneic cardiac transplantation, as currently practiced, is far from ideal if one compares the overall health of transplant recipients (i.e. incidence of infection, malignancy, drug toxicity, chronic vasculopathy, growth failure, etc.) to the health of infants and children of the same age. If allogeneic tolerance could be safely and reliably induced in the newborn, allowing immunosuppressive therapy to be withdrawn, the overall health of transplant recipients

might approach that of normal individuals and allotransplantation would then provide the ideal solution. In that setting, we might view a porcine cardiac transplant as a welcome bridge to the ideal human cardiac allograft. Further, if xenogeneic tolerance could be safely and reliably induced we might be persuaded that xenografts, which can be planned, potentially from the moment of birth or even before, might well be preferred over allografts, the function of which is less than certain. But, we know of no evidence that allogeneic tolerance can be safely and reliably induced in adults or in newborn humans, aside from tolerance to blood group antigens.

The measures now used to attempt induction of tolerance in mature individuals - removal of the thymus and possibly thymus xenotransplantation **(17-19)**, blockade of co-stimulation and immunosuppression regimens and modifications of pigs - extend the survival of some experimental xenografts in non-human primates from months to beyond a year **(20, 21)**, but do not induce tolerance. Indeed, despite decades of work and much progress toward the goal, a safe and effective approach to induction of tolerance in non-human primates toward porcine organs or tissues has not been devised **(22)**. However, Cooper and colleagues reason that existing measures might be successful if applied in newborn individuals with less experienced, more forgiving immune systems. We agree. And, we would add that the function of allogeneic or xenogeneic hearts implanted in tolerant recipients might well be better than the function of hearts transplanted in recipients treated with immunosuppressive agents (that potentially compromise function of kidneys or liver if not the heart). But, we would also add that the more pertinent question is whether overall health would be improved by measures that would decimate the immune system at birth and rebuild it using xenogeneic epithelial cells to select a T cell repertoire that must thereafter protect against microorganisms and toxins presented by self-MHC. We are skeptical on that point and we consider it highly unlikely that a rigorous answer will emerge from the limited number of xenografts performed in newborn non-human primates. That is why we agree when Cooper and colleagues state that presently they would not undertake clinical cardiac xenografts in newborn infants today, recognizing that such agreement favors other non-transplant approaches in the race to conquer congenital heart disease and cardiomyopathies of the newborn.

Some approaches to the "cure" of cardiac failure in the newborn

The surgical approaches presently available to infants born with severe cardiac disease, are far from ideal. The hypoplastic left heart syndrome is relatively common and is generally treated by staged palliation and reconstruction, as first described by Norwood in 1980 **(23)** and as one of us (REC) reviewed **(16)**. Reconstructive procedures continue to evolve and newer approaches are debated today **(14)**. However, despite these improvements, physiology is never fully restored and palliation ultimately fails in ~35% **(15)**. When failure occurs, the patient must undergo cardiac allotransplantation but in this setting transplantation poses a significantly greater risk **(24, 25)** because the recipients are less healthy and often sensitized and the anatomy is compromised **(16)**. While transplantation might be preferred as a primary treatment **(16, 26)**, newborn patients with univentricular hearts are generally not offered transplantation and the few hearts of suitable size are directed to infants with conditions such as congenital cardiomyopathy which cannot be treated by reconstruction. .

When transplantation is performed as a primary treatment, the outcomes are often quite good. Hearts transplanted in newborn recipients grow with the infant and unlike palliative surgery can provide normal physiology. Although rejection can compromise the function and survival of cardiac transplants in newborn recipients, chronic rejection is notably less prevalent than in hearts transplanted in older children and adults **(16, 27)**. The recipients of cardiac transplants must of course take immunosuppressive drugs for life and hence suffer heightened age-adjusted risk of infection and malignancy **(28)**, but these risks are also experienced by the ~35% of those who undergo palliative surgery and then require transplantation for rescue. Still, because suitable donors are scarce, less than 100 cardiac transplants in newborn infants can be performed annually in the United States and ~25-35% of newborns awaiting transplantation die before a heart becomes available **(29, 30)**. Accordingly, Cooper and colleagues join others **(31, 32)** who propose that xenotransplantation could potentially address this agonizing challenge and further advise that advances in the genetic engineering of pigs and the relative ease of inducing tolerance in the newborn (aided by removal of thymus in conjunction with cardiac

surgery) might bring the solution much closer potentially even averting need for ongoing immunosuppression. Whether one agrees or disagrees with Çooper's proposition, much can be learned by considering several of the details.

Graft acceptance early in life

For more than a century, experimental biologists have observed that fetuses and newborn individuals can in some circumstances accept grafts of normal or malignant tissues from other individuals that mature animals do not **(8)**. Acceptance of foreign cells by a fetus may reflect immune incompetence rather than tolerance but the presence of foreign cells from early in life can spontaneously induce tolerance. Indeed, the observations that dizygotic twins of cattle can have spontaneous hematopoietic chimerism **(33)** and that each of a pair of cattle twins, whether dizygotic or monozygotic, would accept grafts of skin from the other twin but not from unrelated cattle **(34)** led to the concept of immunological tolerance. The observations also led to the idea that one might deliberately introduce foreign cells in a fetus or newborn to generate a condition in which the recipient of the foreign cells would later accept a graft from the source of the foreign cells **(35)**(see **(36, 37)** for review).

Fetuses and newborn individuals also can in some circumstances spontaneously accept grafts from xenogeneic sources **(8)**. Fetal sheep, dogs and pigs can accept human hematopoietic cells and the cells can be found at various levels months or years after birth **(38-40)**. Whether the introduction of xenogeneic (human) cells in fetal pigs generates robust xenogeneic tolerance is not clear but it can induce source-specific immune non-responsiveness that persists long after birth (e.g. human hematopoietic stem cells administered to a pig fetus induces non-responsiveness to APC of the stem cell donor with no loss of responsiveness to third-party APC in pigs tested a year after birth) **(41)**.

In contrast to seminal observations in the mouse, foreign tissues or organs transplanted into newborn non-human primates are rejected unless immunosuppression is administered and spontaneous tolerance to foreign tissues does not occur. Rob Michler

transplanted hearts from newborn pigs heterotopically into 5 newborn baboons that were untreated and 5 treated with a "clinically-relevant" regimen of immunosuppression **(42-44)**. The xenografts in untreated recipients were rejected at a mean of ~3 days and grafts in immunosuppressed recipients were rejected at a mean of ~6 days. This experience, albeit limited, suggests Cooper and colleagues are correct to assume a human newborn infant would not spontaneously accept a cardiac xenograft.

A suggestion: Although newborn individuals do not spontaneously accept allografts or xenografts, mammalian fetuses may do so. If a cardiac anomaly is detected *in utero*, one might introduce allogeneic or xenogeneic stem cells or xenogeneic progenitor cells with the hope that the might be spontaneously accepted and later used to repair the defect or improve function. Acceptance of foreign cells delivered to the fetus however does not necessarily impart tolerance **(45)**.

Neonatal tolerance in transplantation

Human newborn infants do spontaneously develop tolerance to the allogeneic blood group antigens expressed in ABO-incompatible cardiac transplants **(3, 46)**. The governance of B cell responses and tolerance to saccharides is incompletely understood. Antibodies specific for some saccharide antigens, such as blood groups A and B and Galα1-3Gal, are usually absent at birth **(2, 3, 42, 46-48)** but after months or years appear spontaneously in all immune-competent individuals who lack the corresponding antigen **(49)**. Foreign saccharide antigens or cells expressing those antigens do not elicit anti-saccharide antibodies in newborn infants and therefore organs bearing those antigens are not subject to hyperacute or acute antibody-mediated rejection **(47, 50)**. Whether absence of these antibodies in the newborn reflects tolerance, i.e. antigen-specific non-responsiveness or a general inability to respond to saccharide antigens is not known. Therefore, the initial acceptance of an organ across a blood group barrier (including Galα1-3Gal) cannot be taken to reflect tolerance. Months or years later, however, the recipient of an ABO-incompatible heart transplant produces antibodies against blood group antigens not present in the graft or in their own blood and then, the absence of a B cell response to antigens in the graft and demonstrable response to allogeneic saccharides

fulfills the definition of tolerance **(3, 46)**. Since B cells specific for saccharide antigens turnover continuously, absence of a response to saccharides in the graft indicates that tolerance reflects an ongoing process. Why older recipients of ABO-incompatible grafts do not also experience this ongoing process and develop tolerance to the foreign saccharides in their grafts is unclear **(51)**. Various testable explanations might be proposed **(52, 53)**.

Whether or not tolerance to foreign saccharides develops, non-saccharide antigens pose the greater challenge in transplantation. Indeed, it was this challenge that identified histocompatibility antigens and the dramatic experiments overcoming that challenge that identified tolerance **(36)**. Still, when one considers applications of neonatal tolerance, it is wise to recall that the original work on induced tolerance in allotransplantation revealed that only ~10% of newborn mice given a mixture of cells from an allogeneic strain would later accept skin grafts from the same allogeneic strain of mice **(35, 54)**. Subsequent work by others **(55)** and by one of us (LJW) **(56, 57)** successfully induced allogeneic tolerance in nearly all mice of some strains but failed to induce tolerance in mice of other strains, the genetic background, H-2 and certain minor antigen incompatibilities being key determinants. What factors would govern tolerance and graft acceptance in human newborns is unknown.

Because tolerance to non-saccharide antigens, e.g. MHC-encoded antigens and minor antigens, expressed by foreign tissues does not develop spontaneously after transplantation in the newborn period, newborn recipients of cardiac transplants always receive immunosuppression. Therefore, Cooper and colleagues are wise not to rely on spontaneous newborn tolerance to sustain cardiac xenografts in newborn recipients. Instead, they propose measures such as thymus xenotransplantation and blockade of co-stimulation that are being pursued for induction of xenogeneic tolerance in mature individuals **(19, 22, 58)**, reasoning, the measures would be more effective in newborns than in adults.

A suggestion: Characteristics that make the immune system of the newborn more amendable to induction of tolerance and more forgiving of a transplant, make the newborn more susceptible to infection. Given the daunting barriers to induction of xenogeneic tolerance in mature individuals **(22)**, toxicity rather than efficacy in early infancy will likely prove limiting. Therefore, where possible, we would pursue tolerance by introduction of foreign cells in the fetus, since doing so preserves host defense.

The source of hearts for cardiac xenografts in newborn infants

As sources of hearts for transplantation in newborn infants, Cooper and colleagues propose using pigs with various genetic manipulations, but especially pigs with targeted disruption of enzymes that synthesize three saccharides recognized by human natural antibodies. Long-term survival achieved using hearts from pigs lacking one or more of these saccharides, including "triple KO pigs" proposed for use, has generated much excitement, seemingly bringing xenotransplantation to the verge of clinical application (see **(59)** for review). Still, some might wonder why in an approach to induction of tolerance in neonates would omit the only types of substances proven to induce tolerance in human neonates **(3)**. Notwithstanding this contradiction, we think there is an interesting and potentially fertile logic to the proposal.

Although loss of tolerance to autologous-blood group A or B saccharides has never been described and newborn recipients of ABO-incompatible cardiac transplants exhibit enduring tolerance to blood groups of the donor **(60-62)**, there is as yet no proof that the tolerance that develops to allogeneic saccharides in recipients of cardiac transplants is as robust as tolerance to self. Indeed, some recipients of ABO-incompatible cardiac transplants performed after the newborn period have low concentrations of antibodies that bind to donor-type erythrocytes **(51)**. Work by one of us (LJW) indicates at least some of these antibodies recognize determinants not present in the graft and hence are not subject to the processes that engender tolerance **(61)**. On the other hand, as we discuss in detail elsewhere **(53, 63, 64)**, absence of antibodies in serum against donor antigens, particularly blood group antigens cannot be taken as proof of tolerance because organ

transplants can absorb enormous amount of antibody when accommodation is present **(53, 63)**.

Why is tolerance to foreign saccharides readily induced in infants but not in older individuals? The principle mechanism usually considered is that immaturity of B cells in newborn infants favors development of tolerance over immunity when the cells confront foreign saccharides (analogous to development of tolerance rather than immunity when newborn mice confront by foreign histocompatibility antigens). While appealing, this explanation does not explain how tolerance to blood groups is maintained as new B cells are produced in mature individuals. Nor can a developmental mechanism alone explain anecdotal reports of development of anti-recipient isohemagglutinins in cord blood transplantation or the transient appearance and then loss of isohemagglutinins in peripheral stem cell transplant recipients **(65)**. Thus, factors extrinsic to B cells probably contribute to the maintenance of B cell tolerance and offer possibilities for therapeutic applications in xenotransplantation.

One factor that could explain the development of tolerance in newborn and responsiveness in older recipients of ABO-incompatible cardiac transplants concerns age- or treatment-related changes in gut mucosa or in gut microorganisms. Cells of mature intestinal mucosa produce plentiful mucin proteins with polysaccharide substitutions, including blood group antigens and mucin-derived polysaccharides have been used as immunogens to generate polyclonal anti-blood group antibodies. Gut bacteria can produce saccharides that cross-react with blood group antigens and these bacterial saccharides have been implicated as the primary stimulus for natural production of isohemagglutinins **(66)**. In principle, either source of antigen could suffice to generate immunity to blood group antigens. However, endogenous cells outside of the gut might release blood group saccharides in sufficient amounts (perhaps as monomer) to induce tolerance and/or compete with polymerized saccharides when antigen-specific B cells begin slowly to be produced. An ABO-incompatible cardiac transplant might also release enough saccharide likewise to induce tolerance. On the other hand, the existing repertoire of B cells specific for blood group antigens would probably require a much

larger amount of saccharide to induce tolerance or to compete with cross-reactive bacterial polysaccharides and this amount probably exceeds the small amounts emitted from the graft.

Another factor concerns the differential impact of polymerized and monomeric antigen on B cells. Polymerized antigen that crosslinks B cell receptors can induce B cell responses without T cell-help (i.e. they are T cell independent) **(67, 68)**. Membrane fragments released from the transplant at the time of surgery might stimulate B cells remote from the transplant (and hence not be subject to inhibition by small amounts of monomeric saccharides continuously released). Since the newborn infant has few if any B cells capable of responding, the transplant procedure and early episodes of rejection do not generate B cells responses and as B cells are later produced they are subject to control by ongoing release of endogenous saccharides. On the other hand, in more mature recipients, polymerize saccharide or membrane fragments released from the graft at the time of reperfusion or during rejection trigger responses of existing B cells. We have postulated this mechanism might explain the evolution of B cell responses in tissue grafts and contribute to accommodation in organ grafts **(52, 53)**.

A third factor that could explain development of tolerance in newborn but not in older recipients of ABO-incompatible cardiac transplants concerns the differences in the durability of delivery of tolerizing antigen to B cells. Persistence of tolerance depends on delivery of antigen in some form to bone marrow and spleen where B cells that generate T cell independent B cell responses mainly reside. Although donor blood group antigens persist for years in cardiac transplants **(3)**, whether the amount or form of this antigen reaching bone marrow and spleen suffices to maintain tolerance is uncertain; whether the amount and form of xenogeneic antigen that would reach B cells of recipients is likewise unknown. In ABO-incompatible transplantation passenger leukocytes provide a potential source of tolerizing antigen and the persistence and renewal of passenger leukocytes from newborn hearts (instead of or in addition to B cell immaturity) might explain the persistence of tolerance after removal of an ABO-incompatible organ transplant **(69)**. The limited capacity for renewal of passenger leukocytes associated with mature organs

might explain absence or loss of tolerance in older recipients (who do not receive organs from newborn donors). Cooper and colleagues might find that organs from newborn pigs are more apt to induce tolerance than organs from mature pigs.

A suggestion: Various factors discussed above might be exploited to limit immunity and perhaps promote development of tolerance in xenograft recipients. Manipulation or engineering of gut bacteria, delivery of antigen in tolerogenic form and/or expression of antigen in stem cells, possibly of recipient origin, might help limit immunogenicity of xenografts or facilitate tolerance without imposing risk on vulnerable infants.

Potential "toxicity" of xenotransplantation

The potential risks of xenotransplantation, such as the conveying of microbial agents, have been discussed elsewhere and require no further mention here. Concerns about infection that once seemingly blocked clinical application of xenotransplantation are much abated **(12)**. However, certain risks unique to xenotransplantation in newborn recipients merit consideration.

The most obvious risk stems from immunosuppressive regimens or approaches to induction of tolerance that limit the ability of a recipient to mount a primary immune response to the microorganisms infants commonly confront. Those who undergo cardiac transplantation in infancy, and hence removal of the thymus, T cell depletion and maintenance immunosuppression have marked contraction of the T cell receptor repertoire and higher levels of human herpesvirus replication than normal individual but suffer no obvious consequences **(7)**. One must be concerned that further measures, such as co-stimulation blockade, might allow these or other common viruses to disseminate or engender pathology. Although thymus transplantation, potentially can correct the defect, as Cooper and colleagues mention, survival of thymus transplant recipients (who do not receive immunosuppression), is no better than survival of infants with severe univentricular anomalies **(70)**.

A more interesting problem is potentially generated by genetic engineering of pigs to eliminate saccharides targeted by the natural antibodies present in mature humans but absent in newborns. Among other functions, saccharide substitutions potentially block the targets of elicited immune responses. Some human proteins with truncated modifications elicit powerful T cell dependent B cell responses and foreign cells expressing modified proteins and lipids are often less immunogenic. This possibility has implication for transplantation as newborn recipients of ABO-incompatible cardiac transplants develop less robust responses to allogeneic HLA than recipients of ABO-compatible cardiac transplants **(60)**. It would ironic to the extreme if understandable zeal to eliminate antigens from organs designed for transplantation inadvertently prevented development of tolerance and increased the need for immunosuppression in newborn recipients of cardiac xenografts.

Another concern, still merely theoretical, is whether any of the genetic modifications undertaken to decrease antigen expression, inflammation, coagulation etc. confer risks in the newborn that would be difficult to appreciate in mature individuals. As elsewhere detailed **(71)**, binding of natural antibodies, activation of complement and initiation of coagulation and thrombosis at a local level prevent colonization and dissemination of microorganisms. Local containment of microorganisms and removal from endocardium is probably much more important in immunologically naive newborn infants than in mature individuals.

Some suggestions: While Cooper and colleagues wait until the time is ripe to transplant hearts from "triple-knockout pigs" into newborn infants, we might suggest they assure the sources of xenografts and treatment regimens are safe and optimized for the newborn infants. We would compare the immunogenicity of proteins and organs from "triple-knockout pigs" with immunogenicity of organs from pigs expressing those saccharides in newborn animals treated with proposed regimens of immunosuppression; not with respect to binding of natural antibodies but as triggers of elicited immune responses. We would compare the baseline and adaptive functions and the durability of "triple-knockout" hearts with those of wild-type hearts. How one might persuade "triple-knockout" pigs to use a

treadmill is beyond our imagination, but some functions can be discerned by echo- and stress-echocardiography. We would test the function and durability of triple knockout hearts transplanted in newborn wild-type pigs, immunosuppressed as Cooper and colleagues have suggested, to make sure these are not limiting. Aside from the proteins with saccharide substitutions, we suspect the hearts of triple negative or αGal-deficient pigs are no more immunogenic or susceptible to injury than wild-type hearts but why not confirm those suspicions (e.g. by testing kinetics of rejection of male-to-female grafts and/or recovery from coronary occlusion). While Cooper and colleagues wait to use these hearts, we hope someone will make sure the hearts are worth the wait.

Potential costs of delaying clinical application of xenotransplantation

On the other hand, there are reasons to resist the temptation to delay clinical application (if not this particular application) of xenotransplantation. As we discuss elsewhere **(59)**, pig-to-non-human primate models may well have reached the limits for predicting the outcome of clinical applications. Put in another way, porcine organs transplanted in humans today might well survive longer and function better than the same organs transplanted in non-human primates. The diagnostic and therapeutic resources (and dollars) that support clinical transplants eclipse by far the resources that support experimental transplants, allowing earlier, more precise and more effective interventions if clinical xenografts were to fail or complications were to arise. Further, immunity generated by xenogeneic organ grafts in non-human primates might also target human products of transgenes intended to overcome incompatibilities of control of complement and coagulation, etc. Therefore, some genetic engineering of pigs that improves the outcome of experimental xenografts in non-human primates might have no benefit in clinical xenografts.

But, what we think could have the greatest impact on the outcome and application of xenotransplantation is not the development of new strategies for immunosuppression or iterations of genetic modification but experience. Given the extraordinary successes of recent years in prolonging the survival of organ xenografts in non-human primates **(13, 21, 72, 73)**, we think the emphasis should be given to identifying the best clinical

setting(s) for early application of xenotransplantation. For reasons we shall explain in closing, we think those settings might not include permanent xenografts in newborn infants with severe cardiac failure, but eventually could very well include bridge transplants.

The benefit of experience

Some important lessons are potentially drawn from reflecting on what might have been the most famous clinical xenograft. The xenograft was performed at Loma Linda University in 1984 in a newborn infant with hypoplastic left heart syndrome, commonly referred to as Baby Fae **(74)**. The xenograft was performed because surgical palliation was unacceptable, a newborn human heart was not available (and had never been transplanted successfully) and death of the patient appeared imminent. Further, there was much experience in experimental transplants of baboon hearts **(75)**.

The cardiac xenograft in Baby Fae functioned reasonably well for more than two weeks (it was the longest surviving heart transplant, allo- or xeno-, in a newborn recipient at that time). However, myocardial function deteriorated, the concentration of cardiac enzymes in blood increased, a biopsy revealed myocardial injury and renal failure ensued after the second post-operative week and progressed leading to death at three weeks. The cause(s) of graft failure and Baby Fae's demise remains a matter of speculation. Much attention has focused on ABO-incompatibility between the baboon (nearly all are blood type A or B or AB) and Baby Fae, who was blood group O **(74)**. However, there are reasons to question the significance of this disparity. First, the histopathology conveyed in original reports suggests prominent vascular damage but immunopathology revealed only sparse deposits of Ig and complement and endothelium of large blood vessels, particularly donor aorta, was unremarkable **(74)**. Second, experience discussed above, suggests that blood group incompatibility of a heart transplant is not likely to cause dysfunction of a heart transplant in a newborn transplant recipient **(46, 47)**. On the other hand, the recipient received blood products that might well have contained isohemagglutinins and these passively transferred antibodies could have contributed to graft injury. However, the delay in onset of dysfunction and injury to >14 days after transplantation would seem

more consistent with the kinetics of dysfunction and rejection observed in concordant cardiac xenografts than with injury caused by natural immunity or passive transfer of antibodies. Further, the outcome of the transplant in Baby Fae might well have been the best outcome that could have been achieved for any cardiac transplant, allograft or xenograft, in a newborn recipient at that time and it is likely that long-term survival and function would have been observed with the immunosuppression regimens and medical support presently used (20 years ago at Loma Linda most concordant xenografts in mature recipients given clinical immunosuppression regimens survived >1year) **(76)**. However, none of the saccharide antigens thought to impair transplantation of hearts from pigs into humans should have been pertinent to the fate of the transplant in Baby Fae. It is nonetheless possible that function and/or recovery from rejection were hindered by yet unknown incompatibilities between baboon and human. That possibility should make us pause before we assume the outcomes of "humanized" pig organs transplanted into baboons faithfully represent the outcome to be expected if the organs were transplanted into human recipients **(59)**.

Toward a solution for cardiac failure in the newborn

In closing we must consider whether in the end an unlimited supply of organs from optimally engineered pigs and the potential for inducing tolerance will eventually make cardiac xenotransplantation the ultimate treatment of severe cardiac failure of the newborn. Despite our enthusiasm about achievements to date in xenotransplantation and a quickening rate of progress we wonder whether other technologies will surpass xenotransplantation as solutions to the problem.

Some think implantable biocompatible devices of suitable size drawn from the shelves of centers that treat cardiac failure in infancy will eventually gain the forefront. We find it difficult to imagine how a device implanted in a newborn could adapt as well as a transplant to variations in activity and to growth, but we are not engineers. Others think a bioengineered heart generated by ex vivo perfusion of various types of stem cells from a patient through a matrix (perhaps a de-cellularized pig heart) will provide an implantable autologous organ **(77, 78)**. If it functioned and adapted and endured like a normal heart,

it would be difficult to fault this alternative, except from the perspective of cost, which would be high.

An approach we would favor, at least from the perspective of cost, is the possibility that an autologous heart might be generated by "in vivo organogenesis" using stem cells harvested from or generated by reprogramming mature cells from the patient **(79-83)**. For some applications, organogenesis might be induced in the patient, but a more conducive environment and safer approach for generation of a heart could be a reverse xenograft in which pluripotent or partly induced human stem cells are introduced into a fetal animal, such as a pig **(59)**. After organogenesis has begun, the primordial organ or induced cells can be transferred back to the patient in whom formation of the organ and vascularization are completed. If still remote, the concept has attracted increasing interest and enjoyed some progress

Successful application of bioengineering or organogenesis for the treatment of cardiac failure in the newborn infant or in older individuals depends on having a safe and reliable way to support cardiac function during the period of months needed for the autologous organ to form. One approach could be performance of a bridge xenograft eventually to be replaced by the autologous organ. Cooper's proposal makes the application of bridge xenografts easier to imagine.

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