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THE IMMUNE SYSTEM IN INFANTS: RELEVANCE TO XENOTRANSPLANTATION

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Abstract

Despite the improvement in surgical interventions in the treatment of congenital heart disease, many life-threatening lesions (e.g., hypoplastic left heart syndrome) ultimately require transplantation. However, there is a great limitation in the availability of deceased human cardiac donors of a suitable size. Hearts from genetically-engineered pigs may provide an alternative source. The relatively immature immune system in infants (e.g., absence of anti-carbohydrate antibodies, reduced complement activation, reduced innate immune cell activity) should minimize the risk of early antibody-mediated rejection of a pig graft. Additionally, recipient thymectomy, performed almost routinely as a preliminary to orthotopic heart transplantation in this age group, impairs the T cell response. Because of the increasing availability of genetically-engineered pigs (e.g., triple knockout pigs that do not express any of the three known carbohydrate antigens against which humans have natural antibodies), and the ability to diagnose congenital heart disease during fetal life, cardiac xenotransplantation could be pre-planned to be carried out soon after birth. Because of these several advantages, prolonged graft survival and even the induction of tolerance, e.g., following donor-specific pig thymus transplantation, is more likely to be achieved in infants than in adults. In this review, we summarize the factors in the infant immune system that would be advantageous in the success of cardiac xenotransplantation in this age group.

Keywords

Antibodies; Immune system; Infants; Pigs, genetically-engineered; Tolerance; Xenotransplantation

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Authors' Contribution

Literature data were collected and reviewed by Mohamed Bikhet, Mahmoud Morsi, Hayato Iwase, Leslie A. Rhodes, Waldemar F. Carlo, and David K.C. Cooper. The manuscript was conceptualized by David K.C. Cooper, Hayato Iwase, David Cleveland, and Hidetaka Hara. The manuscript was prepared, revised and approved by all authors.

Introduction

There is a paucity of available organs for cardiac transplantation, particularly in infants and young children. Ironically, the situation is becoming more acute, as an increasing number of infants are being maintained alive by improvements in medical or surgical care. Annually, in the USA, more than 600 children with heart disease are listed for cardiac transplantation [1]. Congenital heart abnormalities are common, with an incidence of 1 in 100 live birth [2]. Despite improvements in cardiac surgery and therapeutic cardiac catheterization techniques, heart allotransplantation in patients with conditions such as hypoplastic left heart syndrome remains a curative therapy [3–5]. Some survivors of palliative surgery in infancy subsequently develop cardiac failure and ultimately require a cardiac transplantation for survival [6,7]. Unfortunately, by this time the advantages associated with the immaturity of the immune system in infants have been lost, and sensitization to human leukocyte antigens (HLA) may have developed, further limiting transplantation options.

The immune system in infants is relatively weaker than that of adults (e.g., absence of anti-carbohydrate antibodies, reduced complement activation, reduced innate immune cell activity), and thus antibody-mediated rejection to a pig heart is less likely to occur in this age group [8–10]. In addition, the advances made in the genetic engineering of pigs (e.g., triple knockout [TKO] pigs that do not express any of the three known carbohydrate antigens against which humans have natural antibodies). and the ability to prenatally diagnose congenital heart disease, cardiac xenotransplantation could be pre-planned to be carried out soon after birth.

There is considerable evidence that the infant immune system is immunologically less well-developed than in the adult, which therefore lends itself to manipulation [11–13]. For example, West et al. demonstrated that ABO-incompatible heart allotransplantation could be readily achieved in infants [14–16]. Most infants do not have preformed antibodies against AB carbohydrate antigens, and thus hyperacute rejection of an ABO-incompatible graft does not occur after transplantation. In most patients, donor-specific anti-A/B blood group antibodies remain absent or at low levels throughout the lifetime of the recipient. In some cases, B cell tolerance has been induced (e.g., absence of antibody production to blood group antigens) [17,18], providing encouragement that both B and T cell tolerance (e.g., reprogramming T cells to recognize donor antigens as self, through donor thymus transplantation) might be more likely to be achieved in infants than in adults [11,17,19,20].

The field of transplantation is challenging due to complications associated with organ rejection and chronic immunosuppressive therapy, and tolerance is the ultimate goal [21,22]. Strategies for tolerance induction in adults, such as hematopoietic progenitor cell transplantation [23] are unlikely to be feasible in infants due to the toxicity of the pre-transplant nonmyeloablative therapy. Novel therapies are therefore needed for this age group. Because of the unique characteristics of the immature infant immune system, the induction of tolerance may be feasible in this age group [20].

In this review, we summarize the factors in the infant immune system that would be advantageous to the success of cardiac xenotransplantation and the induction of tolerance in this age group.

The immune system in infants

The limited exposure to antigens in the external environment renders the newborn infant's immune system naïve. After birth, there is an exposure to many antigens of the external world and the immune system responds in an attempt to protect the infant against potential infection [24]. At the same time, the infant immune system takes steps to prevent the development of autoimmune disease [25]. Below we will discuss the evolution of the infant immune system after birth

Ontogeny of the innate immune system

Innate immune cells (Table 1)

Neutrophils—Neutrophils are stimulated by granulocyte-colony stimulating factor (G-CSF) just before birth, resulting in infants having higher numbers of neutrophils (1.5–28×10^9 cells/L) than adults (4.4×10^9 cells/L) [25]. However, the function of these neutrophils is impaired due to lower expression of toll like receptor-4 (TLR-4) [26]. Neutrophils in infants demonstrate the following characteristics: (i) 60% lower chemotaxis due to impaired calcium influx [27,28], (ii) lower adhesion due to defective L-selectin, and macrophage-1 antigen (MAC-1) [25,29], and (iii) less phagocytic function due to impaired neutrophil extracellular traps (NETS) [30], all of which make infant neutrophils functionally weaker than in adults [31].

Macrophages—Macrophages are also functionally impaired in infants with lower expression of TLR-4 [32], and have an impaired ability to secrete interleukin-12 (IL-12) and tumor necrosis factor-α (TNF-α) (both of which are pro-inflammatory cytokines responsible for inducing a T helper (Th) 1 type immune response and activation of neutrophils). In contrast, they secrete higher levels of IL-10 (an anti-inflammatory cytokine responsible for downregulation of the Th1-type immune response) [33]. This deviation implies anti-inflammatory (Th2-polarizing) properties of the infant's immune system, which have a protective effect against inflammation [32].

Dendritic cells (DCs)—In infants, plasmacytoid dendritic cells (pDCs) have a very limited ability to secrete interferon (IFN) [34,35], and thus infants have an impaired response to viral infection (e.g., respiratory syncytial virus) [36]. The number of conventional dendritic cells (cDCs) is fewer in infants, with lower expression of HLA class II and CD80/CD86, and lower IL-12 secretion, which impairs the Th1 response [25]. The ratio of pDCs to cDCs in neonates is 3:1 compared to 1:3 in adults. Overall, there is an impairment of the Th1 response, and a more profound Th2 response in infants than in adults [37,38].

Natural killer cells (NK cells)—The cytotoxicity of NK cells in infants is at least three times weaker than in adults [39]. Infant NK cells express less leukocyte immunoglobulin-like receptor-1 (LIR-1), which binds to MHC class I, and higher inhibitory NKG2A receptors.

Therefore, the cytotoxic capacity of human cord blood NK cells, i.e., their capacity to quickly lyse cognate targets without undergoing differentiation, is at least 3-fold lower than in adults [39,40]. They are highly susceptible to inhibition by transforming growth factor-β, and produce limited amounts of IFN-γ [41,42].

Complement (Table 2)—The total amount of complement is lower in infants compared to adults (with 10% to 80% of the adult level for most of its components, except for C7 and factor D). C9 remains low throughout the first year of life which is a pore-forming component of the membrane attack complex (MAC) that is responsible for cell lysis. The level of C1 (the major complement component of the classical complement pathway) remains low for 6 months, unlike C2 and C4 which reach normal levels within 1–6 months [43–46]. In addition to its direct effect on cell lysis, complement plays a role in phagocytosis and B cell modulation. The classical pathway of complement is also impaired in infants due to the low level of immunoglobulin (see below), and thus its effect depends mainly on the alternative, and lectin pathways [24].

Complement regulatory proteins (Table 3)—Complement-regulatory proteins are circulating complement components that are responsible for regulation/inhibition of complement. Some components, e.g., C4b-binding protein, are found at lower levels than in adults, or are even absent [47]. Other regulatory components are at lower concentrations than in adults, e.g., in term neonates, factor H is 64% of adult concentration, and the factor I level is on average approximately only 50% of adult levels [46]. These levels are still lower than adult levels at 6 months after birth [44]. McGreal et al. reported that C1 inhibitor (a regulatory protein of the three activation pathways of the complement system) in term neonates is 62% of adult levels. Collectively, the low levels of complement circulatory proteins balance the low levels of complement-regulatory proteins in infants [46].

Natural antibodies—Natural antibodies in infants are mainly immunoglobulin M (IgM) (produced by B1 cells), the pure definition of natural Abs is that it can be created in the absence of exogenous antigens. Yet, it has also been demonstrated that natural Abs are affected by the presence of exogenous/endogenous antigens, and germline composition, all of which can alter natural Abs repertoire [48]. Isohemagglutinins, such as anti-A and anti-B blood group antibodies, as well as anti-pig antibodies, are natural IgM antibodies. They generally appear at 3–6 months of age [48–50]. Dons et al. showed that there were almost undetectable levels of anti-A/B-incompatible blood group IgM antibodies at one month of age in naïve infant baboons [50] (Figure 1).

Largely through the development of antibodies to galactose-α1,3-galactose (Gal), the most important xenoantigen, most infants have antibodies to wild-type (i.e., genetically-*un*modified) pig carbohydrate antigens within the first few months of life (Figures 1 and 2) [51]. Knockout of the gene for α1,3-galactosyltransferase, which is responsible for adding Gal to the underlying carbohydrates on pig cells, results in reduced antibody binding to pig cells (GTKO pigs) [51] (Figure 2). Binding of infant human antibodies to triple-knockout (TKO) pig cells (that do not express any of the three known carbohydrate xenoantigens) is reduced even further (Figure 3). Indeed, almost no infants develop antibodies to TKO pig cells during the first year of life [52] (Figure 3).

One observation made in our own laboratory questions the assumption that natural antibodies in infants are T cell-independent. Dons et al. provided evidence that anti-pig (mainly anti-Gal) IgM antibodies rose after 3 months of age in naïve infant baboons. In contrast, if T cell costimulation was blocked by the administration of an anti-CD154 monoclonal antibody (mAb), then anti-pig IgM antibodies remained low, but gradually rose after the discontinuation of anti-CD154mAb [50] (Figure 4). When the infant baboon was treated with CTLA4-Ig (instead of an anti-CD154mAb), anti-pig IgM antibodies increased slightly *before* the discontinuation of CTLA4-Ig, suggesting it was less efficacious in this respect. This observation that natural antibodies are not T cell-independent correlates with observations made by Cretin et al. in a mouse model [53].

These observations suggest that, with regard to TKO pig xenotransplantation in infants, there is a 'window of opportunity' (that lasts virtually throughout the first year of life) in which no natural anti-TKO IgM and IgG pig antibodies are present. Additionally, the activation of complement and innate immune cells is weak, thus negating or minimizing the risk of early antibody-mediated rejection [54].

Ontogeny of the adaptive Immune system (Table 4)

The adaptive immune system has two arms – (i) cell-mediated immunity, and (ii) humoral immunity [24,55]. The exposure to antigens is very limited during fetal life, and thus, at birth, the adaptive immune system is still naïve, with a more profound response toward T helper 2 (Th2) cells [56].

T cells—T cell precursors can be found in the liver as early as 7 weeks of gestation [27], but they do not express any surface proteins at this time. During the early stages of development, they are divided into two main groups – (i) α/β T cells, and (ii) γ/δ T cells [25]. The α/β cells migrate to the thymus, where they complete their maturation [57]. The γ/δ cells do *not* migrate to thymus, but instead secrete cytokines to stimulate adaptive immunity, playing an important role in protection against some microbial infections (e.g., Mycobacterium tuberculosis, Listeria monocytogenes, and Brucella abortus) [25,58]. At birth, $CD4^+$T cells and $CD8^+$T cells can be detected in cord blood, contributing approximately 25% of the total lymphocyte count, with $CD4^+$T cells outnumbering $CD8^+$T cells. Most of the T cells in infants are naïve T cells [57,59].

$CD4^+$ T helper cells (Th cells)—The cytokine profile in infants is different from that in adults. Infants have higher levels of anti-inflammatory cytokines (e.g., IL-4, IL-5, and IL-10) and lower levels of pro-inflammatory cytokines (e.g., IL-2, TNF-α, and IFN-γ), which makes the T helper response deviate towards a Th2 (anti-inflammatory) immune response [38,55]. Infantile T helper cells also have a limited ability to express CD40 ligand (CD40L, CD154), which plays an important role in activation of the B cell response [60,61].

T regulatory cells (Tregs)—There are numerous subtypes of T regulatory cells (Tregs), including natural Tregs (nTregs) ($CD4^+CD25^+Foxp3^+$) and induced Tregs (iTregs) [62]. They all have suppressive effects on other T cells and play an important role in protection against autoimmune disease [63,64]. nTregs are present in relativity high numbers in cord blood and

neonatal lymph nodes. They play a major role in the development of central tolerance [65,66]. There is inconsistent evidence regarding the presence of iTregs at birth, but it is clear that the peripheral immune system of neonates has a strong tendency to become tolerogenic upon antigenic stimulation. Whether this is related to the presence of nTregs or a mix of both nTregs and iTregs is still debatable [67].

T helper 17 (Th17) cells—T helper 17 (Th17) cells play an important role in protection from infection. However, its high inflammatory potential through IL-17 can also harm the graft and cause rejection [68]. de Roock demonstrated that expression of Th17 is nearly absent in neonates [69]. There is a reciprocal development pathway between Th17 and Tregs [70]. In newborns, there is an impaired production of pro-inflammatory cytokines, which allows for the dominance of Tregs over Th17 cells, leading to the deviation of the Tregs-Th17 axis towards Tregs. This in turn might help in the induction of tolerance due to the suppressive effect of Tregs [20].

$CD8^+$ cytotoxic T cells—Cytotoxic T cells are responsible for defense against viruses and intracellular pathogens [71]. In infants, they are not as effective as in adults, due to a defective secretion of IL-12 by antigen-presenting cells and lower expression of CD80/CD86 [72,73].

B cells—Early in gestation (7–8 weeks), B cell progenitors are found in the liver. Halfway through gestation, B cell lymphopoiesis takes place in the bone marrow [74,75]. During gestation, most of the B cells are immature B1 cells (and are believed to be T cell-independent), and secrete low-affinity, polyreactive IgM antibodies [59]. At term, these immature B cells represent 50% of all B cells, compared to 12% in adults. They are responsible for early protection against bacteria (e.g., Borrelia hermsii and streptococcus pneumoniae) [76–78].

The antibody-mediated response in neonates is weaker in comparison to adults due to three main factors – (i) T cell expression of CD40L is insufficient [79]; (ii) the lower level of complement, that is in part associated with a weaker antibody response, and (iii) the lymphoid structures in which B cell optimization takes place may not mature during the neonatal period [80–82].

The response to vaccines in infants

The immune response in neonates is deviated towards a Th2 response [83], with a lower Th1 response and a weak humoral response after vaccination. There is, therefore, a defect in the quality and strength of the immune response to vaccination in neonates compared with adults [84,85]. However, there are a few vaccines which generate a strong response in neonates, e.g., Bacillus Calmette–Guérin (BCG), hepatitis B, and oral polio [86]. These vaccines primarily induce a good Th1 response. The reason behind this remains unclear, but might be explained by the ability of the neonate’s immune system to enhance the expression of costimulatory molecules on antigen-presenting cells (APCs), thus mounting a stronger Th1 response [87,88].

The role of the thymus in infants

The thymus is responsible for the maturation of T cells [24,89,90] through positive and negative selection. β chain development in T cells gives rise to the T cell receptor (TCR) [91], while the α chain is associated with the expression of $CD4^+CD8^+$ double-positive T cells (which are the first cells in the T cell developmental pathway to express α/β TCR complexes on the cell surface). The appropriate TCR signaling is crucial for survival of the double-positive cells during this period, and ultimately determines whether developing T cells are positively or negatively selected. Those cells that could not engage will undergo death by neglect [90,92]. The number of naïve T cells remains relatively constant throughout life due to the ability of T cells to maintain peripheral homeostasis [93,94].

The CD31 surface molecule (platelet endothelial cell adhesion molecule or PECAM-1) is present on the T cells exiting the thymus [95,96], enabling CD31 to be used as a marker for recent thymic emigrants (RTEs) [97]. Division of T cells in the periphery results in a lower number of $CD31^+$ naïve T cells with aging. This change is more profound in patients who underwent thymectomy in infancy [98].

Effect of thymectomy in infants (Table 5)

The exact effects of thymectomy in infants remain unclear due to conflicting evidence in the literature. van den Broek et al. reported that the numbers of $CD4^+$ and $CD8^+$ T cells are lower after total or partial thymectomy during the first year of life [99,100]. Other studies claimed that, despite the lower number of $CD4^+$T cells, the number of $CD8^+$T cells remained unchanged [98,101,102]. Mancebo et al. found that there were no changes in the numbers of B cells or NK cells, but the number of neutrophils increased [103]. In contrast, Brearley et al. reported that there was an increase in the number of B cells and in IgA antibodies, with lower numbers of neutrophils [104].

TCR excision circles are small DNA episomes that are present in specific subtypes of naïve T cells, and are used as a marker for recent thymus emigrants [105,106]. As they are not capable of division, they become diluted by the proliferation of T cells, and so (like $CD31^+$ expression) indicate aging of the T cell lineage [100,107,108]. The number of TCR excision molecules is reduced and remains low after thymectomy [109]. Cao et al suggested that the number of TCR excision molecules may correlate with the thymic volume (mass) that remains after thymectomy [110].

Since the early studies of Billingham and his colleagues [111], many studies suggest a role for thymectomy in the induction of tolerance [112–114]. Although the clinical consequences of thymectomy are not certain, some studies suggest there is higher risk for autoimmune disease and a reduced response to vaccines [98,115].

DiGeorge syndrome

DiGeorge syndrome is a primary immunodeficiency disorder resulting from the abnormal development of the third and fourth pharyngeal pouches during embryonic life, resulting in thymic hypoplasia or aplasia, heart defects, and hypoparathyroidism [116]. It can be either partial or complete, and the severity of the disease ranges from no circulating T cells to a

normal T cell count [117,118]. There may be complete athymia, which presents as severe combined immunodeficiency disease (SCID) [119]. There is no relation between the T cell count and the size of the thymus [118].

Most infants with DiGeorge syndrome have a low number of $CD3^{+}$T cells, and most of these cells are exclusively of a memory phenotype (CD4/CD45RO or TCR excision circle-negative) [120]. In selected patients, severe combined immunodeficiency disease can be corrected by successful hematopoietic cell transplantation or by thymic transplantation [119,121,122].

The induction of immunological tolerance in neonates and infants

The induction of donor-specific tolerance is the ultimate goal in organ transplantation, as the recipient would no longer require long-term immunosuppressive therapy [114,123,124]. Tolerance to maternal antigens develops naturally in the fetus, and is retained throughout life.

As an infant, the thymus plays an important role in maintaining tolerance to self-antigens, while trying to defend the body against the exposure to new antigens that occurs after birth, thus allowing the immune system to respond to infection, but avoiding the development of autoimmune disease. This process depends on the development of (i) central and/or (ii) peripheral tolerance [125,126].

Central tolerance can develop in the thymus as T and B cells undergo positive selection, followed by negative selection [127]. However, some T cells escape this process and pass into the periphery where peripheral tolerance may develop through anergy, clonal deletion, and/or the induction of iTregs [128].

The concept of neonatal tolerance and its clinical application is not new [111]. As already discussed, West et al. demonstrated that B cell tolerance to an ABO-incompatible heart graft can be readily induced in infants [11]. However, it is still unknown whether T cell tolerance can be achieved. In xenotransplantation, even if the transplantation of a heart from a TKO pig negated the need for the induction of B cell tolerance to pig carbohydrate antigens (as the recipient would not be exposed to these antigens on the pig heart), T cell tolerance would be required to prevent rejection induced by the presence of protein antigens on the pig cells, e.g., swine leukocyte class I and II antigens [129,130].

In transplantation, many approaches to the induction of tolerance have been investigated, e.g., donor-specific thymic transplantation, mixed hematopoietic cell chimerism, and anergy [131,132]. Almost all studies in large animals, however, have been in adult recipients [133].

In view of the need for intensive pre-transplant immunosuppressive therapy, in our opinion, it is unlikely that hematopoietic cell chimerism will be an approach to the induction of tolerance in infants in the near future. We suggest that tolerance will be more likely achieved by either concomitant donor-specific thymic transplantation or by the induction of T cell anergy (though this latter approach may be associated with loss of tolerance in the event of certain subsequent events, e.g., an infection, in the recipient).

Thymus transplantation

Thymic tissue allotransplantation is already being performed on babies born with DiGeorge syndrome, with good outcomes (around 70% survival), but with some autoimmune complications [134–141]. This is a relatively simple and non-invasive technique as the thymic tissue is injected into the quadriceps muscles of the recipient [134].

In a xenotransplantation model, Lee et al. performed thymus transplantation from pig-to-mouse to induce tolerance, and demonstrated that specific T cell tolerance could be achieved [142]. However, B cells producing antibodies in a T cell-independent manner can still provide a barrier [143]. In large animal models, the transplantation of vascularized thymic tissue increases the success of the induction of tolerance [19,144–146]. Fudaba et al. demonstrated that positive selection, and peripheral maturation of T cells are more efficient when combining thymus transplantation with injection of recipient thymic epithelial cells into the donor thymus at the time of recipient thymectomy [147].

We have suggested that, in neonates or infants, the transplantation of donor-specific thymic tissue (using the Markert technique) at the time of pig heart transplantation (following T cell depletion by thymectomy and the administration of anti-thymocyte globulin) may well induce T cell tolerance [7,54].

Anergy

T cell anergy, in which the recipient lymphocytes are functionally inactivated, may result following the absence or suppression of T cell costimulation (the second signal). Tolerance develops through clonal anergy [148], in which T cells encounter donor antigens when the co-stimulatory signal is deficient. In the absence of costimulation, the T cells enter a state of anergy (allowing a transplanted graft to survive), but this state might be reversible, thus threatening the ‘breaking’ of the state of tolerance [149].

Costimulation blockade is key to the induction of anergy, but other factors may play important roles, including suppression of the mTOR pathway, which can be achieved by drugs such as rapamycin [150]. Although blockade of the CD28:B7 costimulation pathway was considered to be most important in the context of anergy, the CD40:CD154 pathway is gaining increasing attention [151]. This approach has been successful in a mouse allotransplant model [152], but requires further investigation in nonhuman primates and humans [153,154].

Comment

For many neonates and infants with complex congenital heart disease, an immediate heart transplant remains one of the most potent therapies available, and is associated with good survival [155]. Given the need for hearts in this age group, we suggest that clinical cardiac xenotransplantation should initially be considered either (i) as an early bridge to allotransplantation in newborns with life-threatening congenital heart disease, or (ii) as an equivalent to allotransplantation. The excellent long-term outcomes of ABO-incompatible heart allotransplantation in infants provide encouragement that, with an effective tolerance-inducing regimen, both B and T cell tolerance to a cardiac allograft may be achieved [24,54].

The lack of availability of hearts from deceased human donors remains a major barrier to allotransplantation. Xenotransplantation using genetically-engineered pigs as sources of organs offers a realistic alternative, at least as a bridge until a suitable cardiac allograft becomes available. The several advantages of xenotransplantation have been summarized previously [156,157].

The advantageous immunological factors that contribute to the success of heart transplantation in infants, and are likely to facilitate the success of xenotransplantation, are summarized in Table 6. Pre-transplant thymectomy is considered an additional beneficial factor.

We hypothesize that the induction of tolerance to a transplanted pig heart may be possible using a combination of (i) pre-transplant T cell depletion and thymectomy, (ii) a genetically-engineered pig as the source of the heart, (iii) costimulation blockade-based immunosuppressive therapy, and (iv) donor-specific pig thymic transplantation [54]. The possibility of achieving a state of tolerance is greater in infants than in adults due to the relatively immature immune system in infants. Even if tolerance cannot be achieved, prolonged survival of a genetically-engineered pig heart in a recipient receiving maintenance immunosuppressive therapy would enable the heart to be employed either as a short-term ‘bridge’ to allotransplantation, or as destination therapy [54].

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Abbreviations

Gal	galactose-α1,3-galactose
GTKO	α1,3-galactosyltransferase gene-knockout
IL	interleukin
TCR	T cell receptor
TLR	Toll like receptor
TNF-α	tumor necrosis factor-α
TKO	triple gene-knockout

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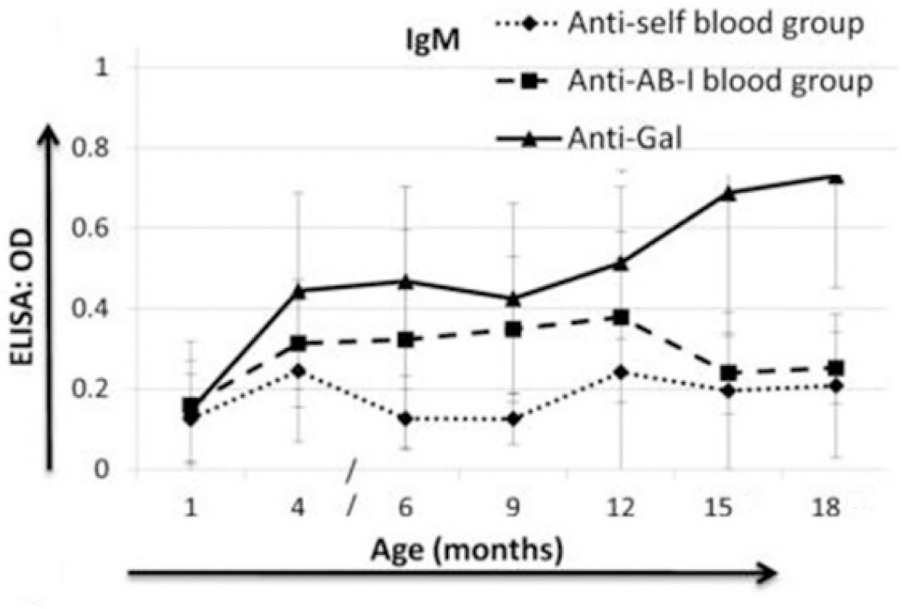

Fig. 1:

IgM levels (mean ± SD) of anti-self A/B blood group (auto) antibodies, anti-A/B-Incompatible blood group (anti-AB-I) antibodies, and anti-galactose-α1,3-galactose (Gal) antibodies, in naïve baboons (n=6). There were undetectable IgM levels of anti-AB-I blood group antibodies, and anti-Gal antibodies at one month of age in naïve infant baboons, comparable with anti-self (optical density<0.2). Thereafter, anti-AB-I IgM increased slowly and anti-Gal IgM rose more rapidly with increasing age. (Reprinted with permission from Dons et al. 2012.)

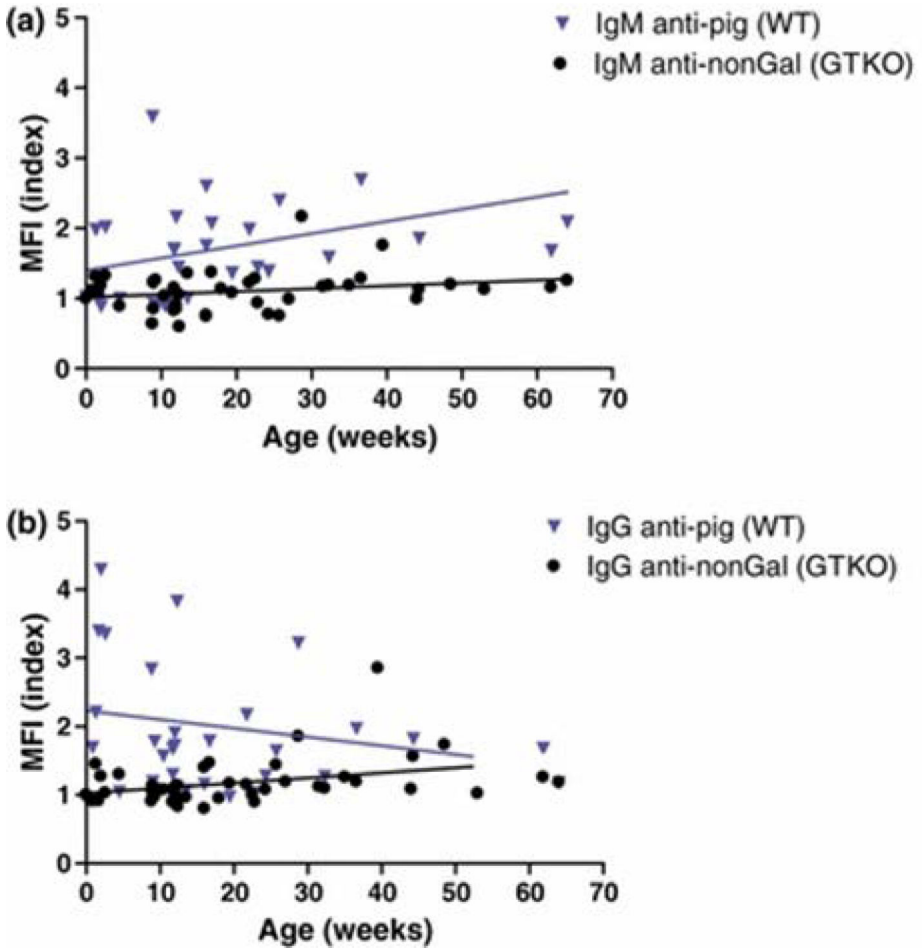

Fig. 2:

Binding of infant human (n=42) serum antibodies to WT and GTKO pig PBMCs. (MFI index = mean fluorescence intensity of the serum sample divided by the MFI of the isotype control sample).

(a) Distribution of IgM reactivity against WT and GTKO PBMCs during the first year of life. Correlation of MFI index with age of each group is indicated by a line (versus WT, p=0.073, r=0.316; versus GTKO, p=0.129, r=0.238). There is a slow increase in IgM directed to Gal antigens on cells from wild-type (WT) pigs, but little increase in IgM to nonGal antigens.

(b) Distribution of IgG reactivity against WT and GTKO PBMC. Correlation of MFI index with age of each group is indicated by a line (versus WT, p=0.381, r= −0.158; versus GTKO, p=0.021, r=0.356). The high level of IgG against Gal antigens on wild-type (WT) pig cells at birth is almost certainly related to the presence of maternal IgG (that crosses the placenta) in the neonate. This falls rapidly after birth. IgG directed to nonGal antigens slowly increases throughout the first year of life. (Reprinted with permission from Rood et al. 2007.)

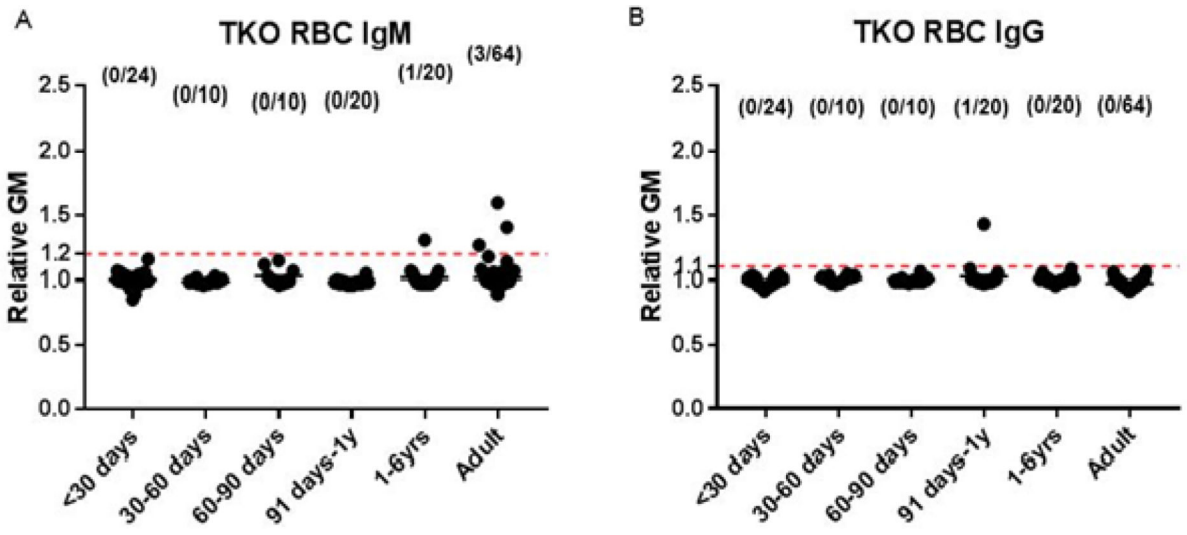

Fig. 3:

Serum IgM **(A)** and IgG **(B)** binding to TKO pig red blood cells (RBCs) in human neonates (<30 days of age) (n=24), infants (1–12 months of age, n=40), children (1–6 years of age, n=20), and adults (n=64). (The dotted line represents the lowest limit of detection, indicating no binding.) **(A)** There is no binding of IgM to TKO pig RBCs during infancy, and only one infant demonstrated any IgG binding. Very few children and adults had any IgM to TKO pig RBCs. (B) Only one infant demonstrated any IgG binding to TKO pig RBCs, and no children or adults demonstrated any binding. (Reprinted with permission from Li et al. 2019.)

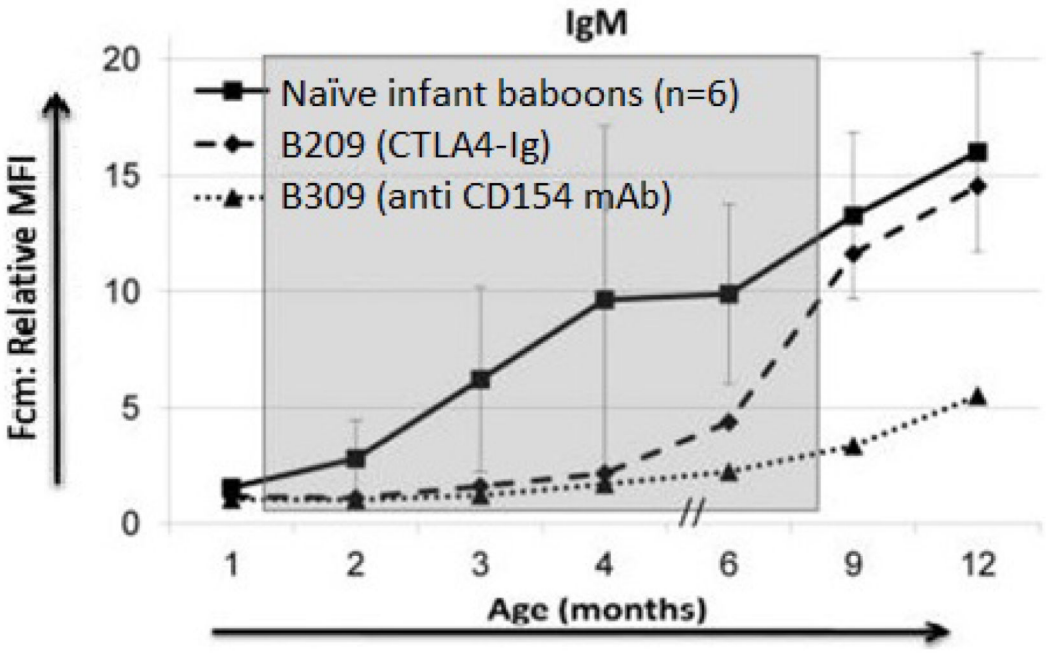

Fig. 4:

Anti-pig IgM antibody levels in baboons with or without the administration of anti CD154mAb or CTLA4-Ig. (The shaded area indicates the period of time when immunosuppressive therapy was being administered to B309 and B209.) (Reprinted with permission from Dons et al. 2012.) In naïve, non-immunosuppressed infant baboons (n=6), after 3 months of age, anti-pig IgM antibody levels rose significantly and continued to increase at 6, 9, and 12 months of age. In contrast, anti-pig IgM antibody level in an anti-CD154mAb-treated infant baboon (B309) remained very low while immunosuppressive therapy was being administered, but rose after discontinuation of the anti-CD154 mAb. In a CTLA4-Ig-treated infant baboon (B209), anti-pig IgM antibody began to increase before discontinuation of CTLA4-Ig.

Table 1:

Innate immune cell markers and function in infants, and comparison with adults

Type of cells	Differences from adults	References
Neutrophils	Higher numbers (13×10^9/L) just before birth, and return to adult levels within 72 hours.Lower adhesion, chemotaxis, and phagocytosis. Lower TLR-4 expression.	26,30
Monocytes/Macrophages	Lower phagocytosis. Lower TLR-4 expression.Lower IL-12 and TNF-α secretion.	32,33
Dendritic cells(DCs)	Lower numbers.Lower HLA class II and lower CD80/CD86 expression.Lower IL-12 secretion.	37
Natural killer cells(NK cells)	Higher numbers.Lower IFN-γ secretion.	40,42

HLA = human leukocyte antigen IFN = interferon IL = interleukin TLR = toll like receptor

Table 2:

Complement components in infants, and comparison with adults

Components	Infants (% of adults)	Time to reach adults levels	References
C1q	65%	1 year	44
C2	95%	6 months	44
C3	70%	1 year	44
C4	73%	6 months	44
C5	65%	6 months	44
C6	65%	6 months	44
C7	Same level as adults		43
C8	Low	6 months	43
C9	10–30%	1 year	43
Factor B	70%	6 months	44
Properdin	25%	1 year	46,47

Table 3:

Expression of circulating complement-regulatory proteins in infants, and comparison with adults.

Components	Infants (% of adults)	Time to reach adults levels	References
C1 inhibitor (C1INH)	60%	1 month	45
Factor H	60%	1 year	46
Factor I	50%	1 year	46
C4 binding protein (C4BP)	Very low	1 year	47

Table 4:

Adaptive immune cells in infants, and comparison with adults

Type of cells	Difference from adults	References
T helper 1 (Th1) cells	Lower CD40 ligand (CD40L) expression (lower B cell response). Lower IFN-γ secretion.	55,60
T helper 2 (Th2) cells	Higher anti-inflammatory cytokine (IL-4, IL-5, and IL-10) secretion.	38
Cytotoxic T (Tc) cells	Lower cytotoxic function due to lower CD80/CD86 expression and lower IL-12 secretion by antigen-presenting cells (APCs).	72
T regulatory cells (Tregs)	Higher numbers in human cord blood (~12% of $CD4^+$ T cells) and neonatal lymph nodes (~8%).	65
B cells	Lower B1 cells (50% of B cells at birth), with higher IgM secretion. Lower CD40 expression.	77

Table 5:

Effect of thymectomy in infancy

Type of cells	Effect on number of cells	References
Natural killer (NK) cells	No change	103
$CD4^+$ T cells	Lower	27,99
$CD8^+$ T cells	Lower No change	27,99,101
T regulatory cells (Tregs)	Lower	110
B cells	Increase No change	103,104

Table 6:

Factors responsible for the relative weakness of the immune system in infants

Cells	Function
Macrophage	Defective secretion of pro-inflammatory cytokines IL-12 and TNF-α
Natural killer (NK) cells	Three times weaker cytotoxic function than in adults
Complement	Lower complement levels, and functionally weaker than in adults
Natural antibodies	Few present until 3–6 month of age
T helper cell response	High anti-inflammatory cytokine profile (IL-4, IL-5 and IL-10), Th2>Th1
Cytotoxic T (Tc) cells	Defective secretion of IL-12
T regulatory cells (Tregs)/Th17	Absent Th17, and more abundant Tregs, that are responsible for T cell suppression
B cells	Defective CD40 expression, and immature B cell response