Xenotransplantation: A View to the Past and an Unrealized Promise to the Future

Luis H. Toledo-Pereyra and F. Lopez-Neblina

Since the early 20th Century when Emerich Ullman transplanted a pig kidney into the arm of a woman (1902), Princeteau implanted portions of a rabbit kidney into the kidney of a child who was dying of renal insufficiency (1905), Jaboulay transplanted two kidneys from a pig and a goat as donor sources (1906), and Unger implanted a monkey kidney into a human (1910), xenotransplantation has made some strides, mostly related to advanced surgical techniques, improved knowledge of immunological principles, and to steps associated with the development of the most effective immunosuppressive therapy.

Innovative surgical techniques were introduced by Alexis Carrel in the first decade of the 1900s, so that vascular anastomoses could be realized without a considerable amount of thrombotic/embolic problems, long before heparin times. Inasmuch as these advances were soundly characterized, it became evident that the results were far from expected and that the time was not ripe for xenotransplantation.

It took 50 years (1963) before Keith Reemtsma transplanted 13 kidneys from chimpanzees into patients with kidney failure. Remarkably, one patient survived for 9 months before dying from electrolyte imbalance. In the ensuing years, Starzl (1964), Hardy (1964), Cooley (1968), Ross (1968), Barnard (1977), Bailey (1984) and a few others entered this new field with less than satisfactory accomplishments.

The unsolvable barrier of hyperacute rejection required persistence and ingenuity. The recognition of xenoantibodies and their requirement for full depletion, through ex vivo porcine perfusion, plasmapheresis, immunoabsorption and complement inhibition, facilitated important advances in this field. The introduction of accommodation and molecular chimerism has further improved the knowledge of this newly conceived field. Advanced molecular engineering techniques have recently permitted the creation of the clonal Gal-deficient pig by eliminating the $\alpha$-1, 3 galactosyltransferase gene. These discoveries together with better immunosuppression raise hope for the yet unrealized promise of xenotransplantation.

Keywords: Xenotransplantation

First Wave of Xenotransplantation

Attempts at xenotransplantation occurred early in the 20th century. In 1902, Emerich Ullman transplanted a dog kidney into a goat by means of prosthetic tubes for the vascular anastomoses with no indication of urinary secretions [1]. It is possible that he also transplanted a pig kidney onto the arm of a woman [2]. Others place Jaboulay (1906) as the first one to perform a clinical xenotrans-
plant from a pig to a human [3,4]. Even though the results were not satisfactory, it was clear then that solutions were being sought pertaining better means to overcome the vestiges of clinical renal insufficiency. The immunological response had not been considered in the overall planning, as little or no knowledge existed of the allograft and/or the xenograft response. Only decades later did a uniform and systematic approach to the immunology of transplantation become clear. The science and technology of transplantation was too young to offer any meaningful support to the first attempted and heroic procedure using the xenograft model described before. So, the past was beginning here. The past was utilizing a desperate surgical technique that had focused in the use of an animal organ transplanted into a human being without consideration for any added scientific sophistication to the procedure. Why and how Ullman and/or Jaboulay came up with this specific procedure is not clear and remains to be considered in another publication. For now, we are focusing on a review of the clinical and experimental highlights of xenotransplantation.

In the same year, 1902, the legendary Alexis Carrel decided to focus on an effective technique for vascular anastomosis. He developed a series of technical steps, which demonstrated the feasibility of the procedure. His technique was primarily based on a detailed and carefully staged surgical event featuring triangular suturing [5]. For Carrel, at this particular stage, the surgical technique was foremost, not the scientific understanding of the rejection response. His contribution was directly associated with maintaining a patent vascular anastomosis for prolonged periods of time and not with xenotransplantation.

Isolated xenotransplant operations were undertaken by Princeteau in 1905, Jaboulay in 1906 and Unger in 1910 [2, 6]. Princeteau conceived of implanting portions of a rabbit kidney into a child critically ill with uremia. No vascular anastomosis was attempted and the kidney did not become viable [2,6]. Clearly, a vascular supply was required to obtain functional status. A year later, Jaboulay transplanted a kidney from a pig and a goat into patients suffering from irreversible renal failure. Both operations were performed in the arm, anastomosing the renal vessels of the donor organ to the brachial artery and the cephalic vein [7]. The results, as expected, were negative. Several years later, Unger unsuccessfully attempted the transplantation of a monkey kidney into the groin of a patient with severe kidney problems [8]. Failure after failure greatly discouraged the few surgeons still interested in the transplantation of animal organs into humans suffering from irreversible kidney disease.

The Silent Period of Xenotransplantation (1910, 1923-1963)

For more than 50 years, except for the case of Neuhof [4,9] of the possible clinical implantation of a goat kidney, the surgical and medical community entertained no interest in xenotransplantation. The few efforts in transplantation were directed to allotransplantation rather than xenotransplantation, which was associated with dismal results. Still, clinical allografts were being utilized by a small group of clinicians, who understood that rejection was among their most important challenges [10]. In the 1940’s, several cadaver kidney allografts were transplanted, still without satisfactory results [3]. The introduction of the artificial kidney in this decade by Kolff dramati-
ally cleared the pathway for clinical transplantation [2]. Patients who previously could not be protected during periods of kidney dysfunction could now be efficiently treated while awaiting for the resumption of kidney function after transplantation.

In the 1950’s, efforts at clinical allotransplantation were reinvigorated. During this decade no xenotransplant was attempted, mainly due to the recognition of immunological problems as the source of early failures. In 1954, Merrill, Murray and Harrison performed the first long-term successful allograft kidney transplant in identical twins [11]. Their success transformed transplantation. Clinicians and researchers systematically approached the treatment of end stage kidney disease with kidney allografts. Xenotransplantation would recede until obtaining enough cadaver donors for human allografting became a problem.

The Second Wave of Xenotransplantation (1963-1984)

The organized second wave of interest in xenotransplantation began in 1963 [2,3,4]. Resolute surgeons believed they could apply all lessons learned from allografts to the xenotransplant model. After all, several decades had intervened since earlier attempts. In the interim, no clinical or experimental work in xenotransplantation had been reported. Clinicians were anxious to demonstrate that xenotransplantation was feasible at this time, and they utilized all available resources from preliminary experiences with allotransplantation. Transplant surgeons combined their knowledge regarding the best operative technique for kidney transplantation, the optimal management procedures, and the most updated immunosuppressive drugs with clear recognition of the allograft rejection response. The immunological barrier was the principle hurdle in the overall evolution of successful transplantation. Existing knowledge and experience were limited by insufficient technological support and incomplete immunosuppression, which was not clearly recognized at the time.

Faced with a shortage of cadaver organ donors, a problem that persists today, surgeons looked for alternatives. Reemtsma, with innovative spirit and decisive boldness, elected to use chimpanzee kidneys. He published his results with 13 patients in severe renal failure a year later in 1964 [12]. Unexpectedly, one patient survived for nine months with satisfactory results. At necropsy, the transplanted kidney was virtually normal. Starzl, who closely followed Reemtsma, reported a series of seven baboon kidney transplants [13]. Six patients survived from 19 to 60 days with no clear rejection as the primary cause of death. Hitchcock, Hume, Traeger, Millard and Cortesini unsuccessfully attempted the transplantation of animal organs for clinical purposes in the following years [4].

Hardy from Mississippi transplanted a chimpanzee heart, and Cooley and Ross separately utilized a single sheep and two pig hearts, respectively [2]. Rejection, spasm and rigidity were reported as immediate causes of organ failure and death. During the same period, Starzl attempted chimpanzee liver transplants in humans with unsuccessful outcomes [14]. A few years later, Barnard used chimpanzee and baboon hearts as auxiliary organs with dismal results [15]. Bailey transplanted a baboon heart in 1984 in a newborn baby, but complete rejection occurred in 20 days [16]. As optimism faded, successful xenotransplantation became a more distant possibility.
Third Wave of Xenotransplantation  
(1992-present)

Despite the failures, surgeons and clinicians still sought the keys to successful xenotransplantation [2,14]. Patients from Poland and India received one pig heart in 1992 and another in 1996 with equivocal findings [2]. Simultaneously, Starzl transplanted two baboon livers [17] and Makowka transplanted a pig liver [18] into a patient with liver failure. Their efforts, as with xenotransplants, ended in failure. Apart from the early anti-coma effects of the extracorporeal xeno-liver perfusion championed by Abouna [19], xenotransplantation remained peculiarly silent. The moratorium was both explainable and prudent because of the clearly disappointing results attained in xenotransplantation. Except for the single patient of Reemtsma who lived for nine months, all patients succumbed within 70 days [2,4]. The clinical picture proved discouraging, particularly given the considerably improved results with human organ allotransplantation.

Basic Sciences Research: From Cell to Pig

Well-defined research in the field of xenotransplantation did not occur until mid 60’s and previous attempts were isolated and characteristically poorly oriented. Studies to better characterize the rejection response after allotransplantation in small animals began in the 1940’s. Several investigators, among them Najarian and his group [20] demonstrated that circulating xenocellular antibodies accelerated xenograft rejection. Later, other causes for xenograft activation were determined that included the alternative pathway of complement, the complement regulatory proteins and the presence of natural antibodies directed against the Gal α[1,3]Gal [14] (Figures 1,2).

The presence of anti-pig antibodies, the discovery of the target molecule, a galactosamine sugar (Gal), the effect of the enzyme α-1, 3-galactosyltransferase (GT), the demonstration of anti-Gal antibodies in humans, the depletion of anti-Gal antibodies, the administration of certain sugars to block Gal sugars, the inhibition of complement, the use of drugs to prevent rejection in all its forms and the achievement of accommodation have all been intricately pursued as a way to overcome the xenograft response [2,14,21] (Figure 3). Excellent reviews of these findings have been amply covered in regards to the significant developments

---

**Figure 1.** Advances in Xenotransplantation to manage the xenograft response.

**Figure 2.** Further advances in Xenotransplantation to manage the xenograft response.
associated with these works [2,14,21].

The next step was to breed a better pig, one that was transgenic and had the Gal molecule knockout. This difficult task required great ingenuity. The work was fully completed and pigs deficient in Gal were produced even though vestiges of Gal were present and could trigger the rejection response. Another possible solution was also contemplated—developing a pig with a complement regulatory protein that would be anti-complement and protect the organ against xenograft injury [2]. The organs of these transgenic pigs were transplanted into monkeys and baboons. The success was only partial, since the recipient animals rejected their organs days after transplant. Thus, another non-complement origin for the rejection process had to be considered [14,21].

Cloning of Animals for Xenotransplantation and More

The cloning of Dolly in 1997 [2] had some transplanters and clinicians anxious to apply cloning xenotransplantation. Dolly-type technology, however, proved cumbersome and somewhat unpredictable as a practical solution to the problem of donor organ shortages. Improved cloning technique was a prerequisite. Perhaps the nuclear transfer technique for individual cells could refine cloning. Perhaps bone marrow or circulating stem cells could be coaxed to differentiate into mature cells of various organs [22]. Perhaps nuclear transplantation of mitochondrial DNA could be performed without immune destruction [23].

Recent advances on the molecular engineering of pigs have resulted in a smashing success—the first cloned knockout pigs [24]. Immerge Bio Therapeutics produced a litter of four knockouts and PPL Therapeutics delivered five newborn piglets named Noel, Angel, Star, Joy and Mary. The GGTA1 gene deleted in these animals encoded GT, a powerful enzyme able to prevent the synthesis of antigens that elicit hyperacute rejection. By adapting nuclear transfer technology to pigs and refining the homologous recombinant tech-

---

**Figure 3.** Possible mechanisms resulting in xenograft accommodation (Partially modified from Dalmasso, AP Understanding and achieving accommodation, Graft 4:55, 2001)
nology of specific targeted genes, these companies were able to produce highly characterized and cloned pigs successfully [24].

**Where Are We Now Then?**

Significant advances have occurred in xenotransplantation, particularly in the past few years. Incredible methodological problems have been solved. New immunosuppressants have been introduced. Target molecules have been defined, and progress in molecular biology has enabled us to perform sophisticated cloning and nuclear transfer procedures. Now we must continue to provide critical support to the knockout cloning technology. This support should materialize in the next five years, while impatient biotechnology companies can still generate momentum in this potentially promising field of medicine.

**The Next Paradigm: The Ethics of Xenotransplantation**

Can we actually offer a new therapeutic modality, with minimal risk and significant benefit, to patients awaiting transplantation? Will allotransplantation of cadaver organs remain the gold standard of treatment? Does xenotransplantation pose an unacceptable risk to public health? Is the incidence of unknown and potentially uncommon animal infections of considerable risk to humans? Are we ready to begin clinical trials? Does the public need to be educated before xenotransplantation becomes universal? Is it right to raise animals for use in xenotransplantation? These and additional tantalizing questions are prevalent in the minds of people intrigued by the potential promise of xenotransplantation.

A rational process for assessing xenotransplantation should include the following elements:

1. The therapeutic methods should be effective.
2. The public should not be at risk.
3. Society should be extensively educated as to risks and benefits.
4. Regulations and protocols should be established.

And yes, I believe it is ethical to raise animals to help human beings. Today the science of xenotransplantation is moving toward clinical trials that will help define the appropriate application of this therapy.

**References:**

13. Starzl, TE, Marchioro, TL, Pteres, GN, Kirkpatrick, CH. Renal Heterotransplantation from Baboon to Man: Experience with
6 cases. Transplantation 1964; 2:752
21. Cooper, DKC (ed) Xenotransplantation Special Issues in Graft, 4:Issues 1 and 2, January/February-March, 2000, p 6-86 and 94-166