

LA TRANSPLANTATION D'ORGANES

Les débuts de la transplantation rénale

Bien que l'article ci-après ne couvre qu'une trentaine d'années, il fait partie de l'histoire de la médecine de l'UCL. Écrites par le Pr G. Alexandre dans le livre *History of Transplantation : Thirty-Five Recollections* Ed. P.I. Terasaki - UCLA Tissue Typing Laboratory - USA. 1991, les pages 337 à 348 sont reprises *in extenso* ci-après avec l'autorisation de l'auteur et de l'éditeur P. Terasaki.



Le Pr Guy P.J. Alexandre (1934) , chef de service de transplantation rénale et de chirurgie des glandes endocrines : 1973 – 1992.

From the early days of human kidney allotransplantation to prospective xenotransplantation

G.P.J. Alexandre

Introduction

Immediately upon receiving my medical degree (1959) from the University of Louvain Medical School in Louvain, Belgium, it was clear to me that I wanted to become a surgeon. I applied to Professor J. Morelle, Chief of the Department of Surgery at the Cliniques Universitaires Saint Pierre in Louvain, where I was accepted as an assistant.

Professor Morelle was a general surgeon, but he had a special interest and competence in the neurosurgical sciences, which revealed itself as a determining factor in the beginning of the transplantation era at our center.

In his department, it was customary to spend a year abroad during the third year of surgical training. For the most part, the decision as to where to go depended on individual wishes. In my case, this decision turned out to be the decisive factor which influenced my surgical career. It was a consequence of a review I had done on “immunology and transplantation” which focused my interest on the field of transplantation.

I obtained a C.R.B. graduate fellowship for a year of surgical research to be spent in the laboratory for surgical research at the Harvard Medical School in Boston, under the direction of Professor Joseph Murray and in the Department of Surgery of the Peter Bent Brigham Hospital, directed by Professor Francis D. Moore.

The Good Years

It is difficult to describe the state of mind of a young graduate in medicine, arriving in the States with his wife and two babies in September 1961, while speaking imperfect English and having even more imperfect understanding. Fortunately, the friendly people we were extremely lucky to meet on our arrival in Boston, helpfully guided our first steps toward establishing ourselves.

My initial contact with Dr. Murray's laboratory was Dr. Roy Calne. He was packing to return to England and gave me advice on how to treat the dogs that were surviving from his experiments with a functional kidney transplant.

Considerately, Dr. Murray gave me a few weeks to “accommodate” before beginning the real work. At that time, the main goal of research was to find new immunosuppressive drugs and regimens. Regular discussions on how to improve the immunosuppressive effects of BW-57322, the actual name of Imuran, were conducted at Burroughs Wellcome headquarters with Drs. G. Hitchings and Trudy Elion (who also received the Nobel prize since then).

During my stay in Dr. Murray's laboratory, hundreds of dogs received a kidney allograft in an effort to find the best immunosuppressive regimens. These were times when up to five kidney transplants a day were being performed in the laboratory with a team consisting of two technicians and me; still, weather permitting, I was often spared some time to play tennis at midday with my Peruvian technician. It was great ! Everybody was enthusiastic and excited about the results.

One drug combination, azaserine and actinomycin D, gave especially good results, which were reported to the Congress of the AMA at Atlantic City since the entire series of 16 dogs had retained a functioning kidney for more than 20 days, a record in those days.

This drug combination was considered good enough to be used in clinical practice. I was then given the responsibility of preparing the azaserine solution to be administered to the transplanted patients at the Brigham Hospital. With this priceless solution in hand, I could enter the patient's rooms in the evening, often with suspicious looks from the nurses because of what I was doing to their patients. Needless to say, everything had to be explained in great detail ! Looking back at this period of my life, I must confess that it was probably the most enjoyable and the most exciting. It became clear that long-lasting functioning renal allografts could be obtained in dogs with nonspecific immunosuppressive regimens. And if it were possible in dogs, it surely would also be the case in man. I returned to Belgium with this good news to complete my surgical training.

The First Kidney Transplant in Belgium

With some azaserine and actinomycin D in my luggage, the return trip was made in the hope that kidney transplantation could also be started in Belgium. Convincing everybody of the feasibility of the procedure was not an easy task but, fortunately, my department chief supported the idea. He was even more convinced after we had repeated the surgical procedure twice on a cadaver. All that remained was to find a suitable patient and to maintain him in a suitable condition until a kidney could be obtained and transplanted. Since no chronic dialysis apparatus was available in our department at that time, the first patients were maintained with peritoneal dialysis, performed by medical students on a voluntary basis and in 24-hour rotation.

On June 3, 1963, a patient was brought in with a head injury and in profound coma. The patient became completely areactive, had a falling blood pressure despite the administration of vasopressive drugs, and presented all the signs of what Mollaret had described as a "coma dépassé," a notion which has since been anatomically confirmed by Inkvaard.

Professor Morelle, who was quite experienced in neurosurgery, considered the neurological symptoms presented by the patient and took what today could be considered the most important decision of his career: whether to remove a kidney from that patient while the heart was still beating. This procedure was

probably the first transplant ever removed from a heart-beating cadaver. Fortunately, this was long before the days of established ethical committees. The kidney was transplanted without delay. In fact, the kidney was removed by Professor Morelle and his team while another team and I were preparing the recipient in an adjacent room. No preservation fluid was used; the blood contained in the transplant was not even washed away; the graft functioned right away without tubular necrosis; and the patient's serum creatinine was back to normal within a few days. Unfortunately, this patient died from sepsis of the wound after three months. His follow-up, however, took long enough to convince other colleagues that the procedure could be done and that drug immunosuppression was effective.

At the time of this first patient's death, three other patients had been transplanted. The third patient was transplanted with a living donor kidney given by the patient's uncle. That patient's kidney functioned for more than six years as did the kidney of the fourth patient transplanted with a cadaver kidney. With these two long-term survivors, the transplant program at our center was launched. From then on, the number of transplants performed annually has increased progressively and the program has been adapted to the latest progress.

The Birth of Eurotransplant

In 1967, Dr. Jon van Rood presented the idea of selecting the recipient of a cadaver kidney in accordance with the compatibility of the donor and recipient leukocyte antigens. Only a small number of HLA antigens could be determined at that time. Those were the days of the antigens 4a, 4b, 6a, 6b enz. The difficulty, however, was the kidney preservation since the preservation solutions were far less effective; therefore, there was a real need for expediting all the procedures at maximum speed.

Fortunately, Dr. van Rood's laboratory in Leiden, Holland, is not so far from Belgium, and I must confess we received maximum logistical support from the Belgian police corps and the light aviation unit of the Belgian Army. When a donor was available, blood was sent by helicopter or car to Leiden, and as soon as the results of the HLA antigens were known, the kidneys were delivered to the best available match. In the beginning, these exchanges were restricted between Holland and Belgium. For some years, one army helicopter was reserved strictly for these missions and was known in the army as "the kidney helicopter".

When the distance was not too great or a donor kidney became available at night, one of us did the transporting. Dr. P. J. Kestens, well known among us for

his fast driving (presently Professor of Surgery and Chief of the Department of Surgery at our Clinics), once had an auto accident while transporting a kidney at a time when the recipient was already prepared in the operating room. The only way the kidney could arrive safely and in time was for Dr. J.J. Rombouts, who had accompanied Dr. Kestens, to hitch-hike. Fortunately, it did not take too long.

Antilymphocyte Serum “House Made”

After Dr. Thomas Starzl showed that better renal transplantation results could be obtained by treating recipients with antilymphocyte serum, we embarked on the local preparation of horse antihuman antilymphocyte serum. Horses disqualified for duty because of lameness were bought from our National Mounted Police Corps and injected with thoracic duct lymphocytes. These were being collected from recipients of living donor kidneys who, in that period of time, were prepared at our center with a five-day preoperative drainage of the thoracic duct.

The horses were housed at a local farm, and we had the responsibility of injecting the antigenic material ourselves. Fortunately, the farmer was well aware of all the tricks of keeping the horses quiet during the injections ! Still, we were a bit afraid that they could react violently and kick us out of the stall. After immunization, the horses were brought to the laboratory where a horse box had been built, and there, they were bled. Professor G. Sokal, head of the immunohematology laboratory took the responsibility of extracting the antilymphocyte globulins and prepared the vials for injection to the patients. Since the preparation of this ALG was cumbersome and somehow monopolized the laboratory activities, contact was made with Behringwerke Pharmaceuticals (Germany) which took over this preparation which led to the commercialization of the Behring horse antihuman ALG, a very active, well-known product around the world today that has been administered to thousands of transplanted patients. A prospective randomized series at our center clearly demonstrated the benefit of ALG in human renal transplantation, and since October 1976, it has been used prophylactically in all patients at our center.

Dr. J. P. Squifflet joined our staff in 1978. In 1981, he obtained a research fellowship grant at the University of Minnesota in Dr. J. Najarian's department, where he began a PhD program in pancreatic transplantation under Dr. D. Sutherland's supervision. A few months after Dr. Squifflet returned from Minneapolis, the first pancreatic transplant was performed in our department,

actually the first ever done in Belgium, and the beginning of our pancreatic transplant experience.

ABO-Incompatible Transplants

Early in 1981, we accidentally performed an ABO-incompatible cadaver kidney transplant which turned out to be a blood group A1 into an O recipient. At the time the error was discovered, the patient had already been transplanted. Since the graft function was excellent, the patient was heparinized but otherwise received the standard triple immunosuppressive therapy consisting of prophylactic antilymphocyte serum (ALG, Behringwerke, Germany), Imuran, and low-dose steroids.

The follow-up was uneventful until the beginning of the third week, at which time a rejection crisis developed with a sharp increase of the anti-A isoagglutinins which reached a titer of 1/20,000. Antirejection therapy consisted of boluses of methylprednisolone and antilymphocyte serum. Complete control of the rejection crisis was obtained without much difficulty; the isoagglutinin titer decreased and the renal function has remained normal since. The patient is now more than nine years posttransplantation.

The happy ending to this particular case led us toward a renewed interest in the possibility of achieving ABO-incompatible transplants. By discussing the matter with Drs. M. De Bruyère and D. Latinne, respectively Professor of Medicine and Associate Chief of Laboratory in the Immunohematology Department and with Dr. M. Moriau, Professor of Medicine in the Hematology Department at our University clinics, we thought we could use a similar approach to that used in achieving ABO-incompatible bone marrow transplants to prepare recipients for ABO-incompatible living-donor kidney transplants.

The first application of this clinical protocol was the case of a 9-year-old girl with blood group O who was prepared to receive her mother's A1 kidney by preoperative plasmaphereses to get rid of the antidonor isoagglutinins. After the last plasmapheresis, we administered soluble substance A to eliminate the remaining isoagglutinins. The recipient was splenectomized at the time of and immediately prior to grafting. At that time, splenectomy of the recipient was routinely performed in all cases of living-donor kidney transplantation at our center. This was also the case for living-donor ABO-compatible kidney transplantation. Fortunately, the postoperative follow-up of this case was uneventful. Renal function became normal after a few days and has remained so. No rejection was ever noticed. Encouraged by this case, we decided to go on. At that time, the recipients of living-donor ABO-compatible kidney transplants at

our center were being prepared preoperatively with donor-specific blood transfusions (DST). We thought that the recipients of an ABO-incompatible kidney should also benefit from DST but, for obvious reasons, whole blood could not be used. Therefore, it was decided to use donor-specific platelet transfusions (DSPT) instead, since platelets do not contain ABO blood group antigens. The experience with DSPT in ABO-incompatible transplants demonstrated the same disadvantage as using DST in ABO-compatible transplants, ie, the production of sensitization to the donor-specific blood products that prevent the living-donor transplant in $\pm 10\%$ of the cases. Therefore, the use of DSPT was halted.

Since the first five ABO-incompatible cases succeeded without much difficulty, we were confused by the failure of the following three consecutive cases which we attributed to the fact that these three patients were not splenectomized. The reason splenectomy was omitted resulted from a change in our protocol in living-donor kidney transplantation. As explained previously, at the time ABO-incompatible living-donor transplantation was initiated at our center, splenectomy of the recipient was part of the standard protocol being used in this category of patients. Therefore, the decision to abandon splenectomy in living-donor kidney transplantation was taken, irrespective of the ABO-compatibility or -incompatibility of the donor.

After the third consecutive failure, we realized that the only change we had made in our protocol relative to the first five cases was indeed the splenectomy which we had omitted in the last three cases. Although no clear-cut scientific conclusion could be drawn from such a small series, it was decided to reintroduce prophylactic splenectomy in the protocol of ABO-incompatible living-donor kidney transplantation as a standard measure. A more scientific approach would have been to start a randomized prospective study of splenectomy against no splenectomy in this category of patients but due to the three consecutive failures which we experienced, it was felt that such a study was ethically unacceptable.

The observation that isoagglutinins could be allowed to return after transplantation without necessarily producing an irreversible rejection of the transplant surprised us. More disturbing, but even more interesting, is the fact that some patients may carry very high titers of isoagglutinins, much higher than prior to the transplant and still tolerate the ABO-incompatible kidney quite normally, as if the transplant had become indifferent to these antibodies. Meanwhile, we could demonstrate that the ABO blood group antigens have not disappeared from the endothelium of the transplanted kidney. Indeed, if one

takes a biopsy of a well-tolerated ABO-incompatible kidney and challenges this kidney tissue with the patient's own serum in vitro, there is a clear-cut fixation of the patient's isoagglutinins on the endothelium clearly demonstrating that the antigens are still in place. Why these antibodies which react in vitro do not react in vivo is unknown. Dr. F. Bach proposes the word "accommodation" to name this phenomenon whereby the three major components of an immunological reaction, ie, a mixture of antigens complement, and specific antibodies do not react together.

J. Platt proposes several explanations for this phenomenon of accommodation :

- a change in the susceptibility of the graft endothelial cells to injury from the isoagglutinins;
- a change in affinity and/or specificity from the natural antibodies present before grafting;
- a modulation of the epitopes normally expressed by the graft endothelial cells.

From our clinical experience, it appears that a rapid postoperative increase of the antibody titer generally leads to irreversible graft rejection while those patients maintaining a low profile of these antibodies generally enjoy normal functioning transplants.

Therefore, a primary condition to obtain accommodation must be the control of the postoperative production of the natural antibodies during the initial postoperative period. In our ABO-incompatible series of patients, we never observed any humoral crisis of rejection after the third postoperative week, which indicates that probably a critical period exists during which the ABO-incompatible transplant is at risk of acute vascular (humoral) rejection but after which, the transplant behaves in a conventional manner.

Another observation is that in a particular patient, we never observed two successive vascular rejections so that the accommodation may be either produced progressively when the patient does not present any rejection crisis, or the accommodation installs itself at the outset of a well-controlled rejection crisis. Since the majority of patients receiving an ABO-incompatible kidney transplant present one rejection crisis, one may believe that accommodation is brought about in the majority of cases when the patient is coming out of a successfully treated rejection crisis.

Whatever the mechanism of accommodation may be, it is important to realize that it allows successful transplantation across preformed antibodies which opens the barriers of xenotransplantation.

Pig-to-Baboon Renal Xenotransplantation

The major obstacle to xenografting between discordant animal species such as pig-to-baboon or pig-to-man is also represented by natural preformed antibodies (NPA) of the recipient species against the donor species producing acute vascular irreversible rejection of the transplanted organ. There is, therefore, a similarity between ABO-incompatible renal transplantation in the human and renal discordant xenotransplantation since NPA are responsible for the violent vascular rejection phenomenon observed in both models.

Similar to the situation existing in human ABO-incompatible renal transplantation, manipulation of these natural antibodies in such a manner as to avoid the triggering of the xenograft rejection phenomenon is a prerequisite for successful long-term organ xenografting.

Owing to the success obtained in our series of ABO-incompatible renal transplants, we chose to explore the possibility of achieving pig-to-baboon renal xenotransplantation. In doing this, we obtained the full support of W. De Meurichy, Dr. of Veterinarian Medicine at the Koninklijke Maatschappij voor Dierkunde of Antwerpen and Professor A. Dewaele, Chief of the Department of Pathology at the Faculty of Veterinarian Medicine of the University of Liège. We thought that if “the same causes produce the same effects,” then treating a baboon recipient of a pig kidney by the same regimen as a human recipient of an ABO-incompatible renal allograft could permit a breach to the barrier presented by the NPA in the field of xenotransplantation.

In October 1984, the first pig-to-baboon renal xenograft was thus performed in our laboratory for surgical research and to our great satisfaction, this renal xenograft succeeded in maintaining a satisfactory function for 13 days, whereas controls lasted a maximum of 35 minutes to a few hours. The combination of preoperative plasmapheresis with splenectomy and a quadruple immunosuppressive therapy demonstrated its effectiveness in prolonging a discordant renal xenograft far beyond the survival time that had been obtained so far in this type of model.

Although some eminent immunologist told us that this prolongation was nothing more than a “ball chance,” we carried on with the help of Drs. P. Gianello and D. Latinne but chose to use rhesus monkeys instead of baboons. The change in animal species was suggested by financial and logistical considerations. This idea, however, did not turn out to be a very good one. The very small caliber of the vessels caused many difficulties in achieving plasmapheresis in these animals. Therefore, after a series of technical failures, we switched back to our initial idea of using baboons.

To date, a dozen pig-to-baboon renal xenografts have been achieved. In this series, three animal recipients are worthy of more detailed description since they survived 10, 22, and 23 days, respectively. The baboon who lived for 10 days died of a pulmonary infection; the renal function was normal (1 mg %) at the time of death and histology of the xenograft at the time of death was remarkably normal. The two baboons who lived over three weeks presented a normal renal function during the first postoperative days. Each of them presented an acute rejection crisis at the end of the first week, but while this rejection crisis was vascular (humoral) in nature for the first animal, it was strictly cellular and mild in the second one.

This example of an interstitial cellular rejection in a discordant xenograft model is the first of its kind that we know of. It represents an indication that once the obstacle of the humoral barrier is overcome, the better known cellular rejection remains to be avoided, which, according to some reports, could be easier to handle in xenografts than in allografts.

Organ Transplantation in the Future

In a relatively short period of time (30 years) organ transplantation has become a very effective therapy to treat an extensive number of lethal diseases. Patients who were condemned to death only a few years ago return to active life with a well-functioning organ transplant. Double organ transplantation like heart and liver, heart and kidney, heart and lungs, kidney and pancreas, triple organ transplantation like heart, liver, and kidney or even more complex organ transplants like the cluster organ transplants, have become possible with good success.

There is, however, a shortage of organs which will increase even further with the broadening of the indications for organ transplantation and the need for retransplantation in cases of chronic rejection. The challenge for the coming years is clear: we must find a way out of the shortage of transplantable organs while at the same time unspecific and specific immunodepressive means need to be developed and refined.

In my opinion, xenotransplantation is the way to go. We have shown that the humoral barrier is not insuperable; new immunosuppressive drugs are being discovered, which alone, or most probably in combination, will allow for better control of the immunological reaction. Unspecific immunosuppression may be combined with more specific means to produce specific tolerance. These, together with an appropriate preparation of the donor animal and the recipient patient, should allow for acceptance of a xenografted organ in the not too distant

future. Then we will have achieved our goal to transplant all the candidates for organ transplantation without being compelled to register the patients on evergrowing waiting lists.