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Il maiale per alcuni dati peculiari (caratteristiche fisiologiche, dimensioni adeguate, facilità di allevamento, scarso impatto etico, ecc.) sarebbe la specie animale più indicata allo xenotrapianto. Il maggior ostacolo all'utilizzo di organi provenienti da tale specie è data dalla presenza nell'uomo di anticorpi preformati citotossici verso gli antigeni xenogenici e responsabili del rigetto iperacuto. Il modello pre-clinico che più si avvicina alla situazione del trapianto di maiale nell'uomo è dato dal trapianto maiale-babbuino. In questa combinazione il trapianto di rene viene rigettato entro pochi minuti dalla rivascolarizzazione. La strategia terapeutica utilizzata dall'Autore per controllare questa violenta reazione si basa sulla splenectomia associata a cicli di plasmaferesi del potenziale ricevente, seguita dalla terapia immunosoppressiva convenzionale (ciclosporina, steroidi e azatioprina) nel decorso postoperatorio. Questo protocollo è in grado di prolungare la sopravvivenza dell'organo trapiantato fino a 3 settimane. Nonostante il rebound anticorpale post-trapianto, l'Autore ritiene che la presenza di alti livelli di anticorpi circolanti non precluda alla funzione dell'organo e che un probabile fenomeno di «adattamento» immunologico possa instaurarsi nel ricevente ed essere responsabile dell'accettazione del trapianto.

Pig to baboon model in renal xenotransplantation

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The supply of organs is the greatest challenge facing the transplant community today. Waiting lists for organ transplantation are increasing all over the world: in eurotransplant, the number of patients waiting for a kidney is also increasing every year; it it now over 10,000. This increase is the consequence of two major factors: the acceptance of older candidates for kidney transplantation and the need for regrafting after graft failures from chronic rejection.

The situation is worse for patients in need of a heart, liver or lung transplant due to the lack of any valid replacement therapy. As a consequence, too often, patients die before a suitable transplant can be found. As for the candidates for a kidney, the waiting time for many of them may be excessively long when no living donor is available.

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Xenotransplantation would be an ideal solution to the shortage of organs. If and when it will be feasible, waiting lists should no longer exist; most patients could be transplanted before the endstage insufficiency of the diseased organ. Xenotransplantation also offers the benefit of a possible prolonged preparation of the recipient to receive a specific xenotransplant and therefore allows for prospective immunological manipulation of both the donor and the recipient to obtain specific tolerance to the transplanted organ.

The major barrier to discordant xenotransplantation is represented by the preformed natural antibodies (PNA) of the recipient species against the donor species producing humoral rejection of the graft in a matter of a few minutes to a few hours. Jonasson has re-

sumed the problem by saying: "Until the lesions produced by preformed antibodies can be prevented by immunological or pharmacological manipulations of the recipient or of the donor organ, successful xenografting for the long term will be impossible [1]".

Now, there is a condition in man in which preformed natural antibodies may similary produce humoral rejection of the transplant: that is the transplantation of a donor kidney into a blood group ABO-incompatible recipient. The end result of an ABO-incompatible allografted kidney is indeed very similar to that of a discordant renal xenograft. In both cases, recipient PNA react with the donor vascular endothelium to initiate intravascular coagulation with ensuing complete thrombosis of the transplant vasculature.

We have shown however that this cascade of events may be circumvented when recipient's PNA, i.e. the isoagglutinins against the incompatible donor blood group antigen(s) have been eliminated before transplantation by plasmapheresis [2 3]. We have also shown that splenectomy of the recipient prior to or at the time of transplantation is an important factor for success [4].

Sofar a series of 30 ABO-incompatible living donor renal transplants and a series of 7 ABO-incompatible unrelated transplants have been performed in our center in 36 patients since one patient received successively two ABO-incompatible living donor grafts. The overall results are better in the series of related living donor transplants [5 6], the best results being obtained in the 18 cases of mother to child kidneys. In this latter series, only 2 grafts were rejected. The first patients of the series are now over 8 years with a functioning graft.

In all cases, the recipient's isoantibodies return after grafting. At the occasion of a rejection crisis the isoantibody titer may increase sharply and diminish again, although more slowly when the rejection crisis is being controlled. When the increase is too sharp, the transplant may be violently and irreversibly rejected [7].

All long term functioning ABO-incompatible transplants retain their function notwithstanding the presence of specific isoantibodies directed at the ABO antigens of the graft endothelium. The isoantibody postoperative titer may even remain higher than the preoperative one. At this stage an equilibrium has been obtained which Bach proposes to refer to as a phenomenon of "accomodation" (fig. 1).

Owing to the success obtained in our series of ABO-incompatible renal transplants, we choose to explore the possibility of achieving pig to baboon renal xenotransplantation. We choose the pig because it could be a good candidate for a donor species to man. It is ubiquitous, easily bred, even under germ-free conditions; it is a prolific species whose physiology resembles that of human physiology. The baboon was choosen as being the closest species to man which could be used without too much difficulty to mimic a possible future clinical application of pig organ transplantation to the human.

As already pointed out, 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 (PNA) of the recipient species against the donor species producing acute vascular irreversible rejection of the tranplanted organ. There is therefore a similarity between ABO-incompatible renal transplantation in the human and renal discordant xenotransplantation since PNA are responsible of the violent vascular rejection phenomenon observed in both models. Incidentally, the titer of baboon or human agglutinins against pig red cells in the range of the isoagglutinin titers which can be found in the recipients of ABO-incompatible kidneys in the human.

Similarly 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.

We thought that if "the same causes produce the same effects", then treating a baboon recipient of a pig kidney by the same

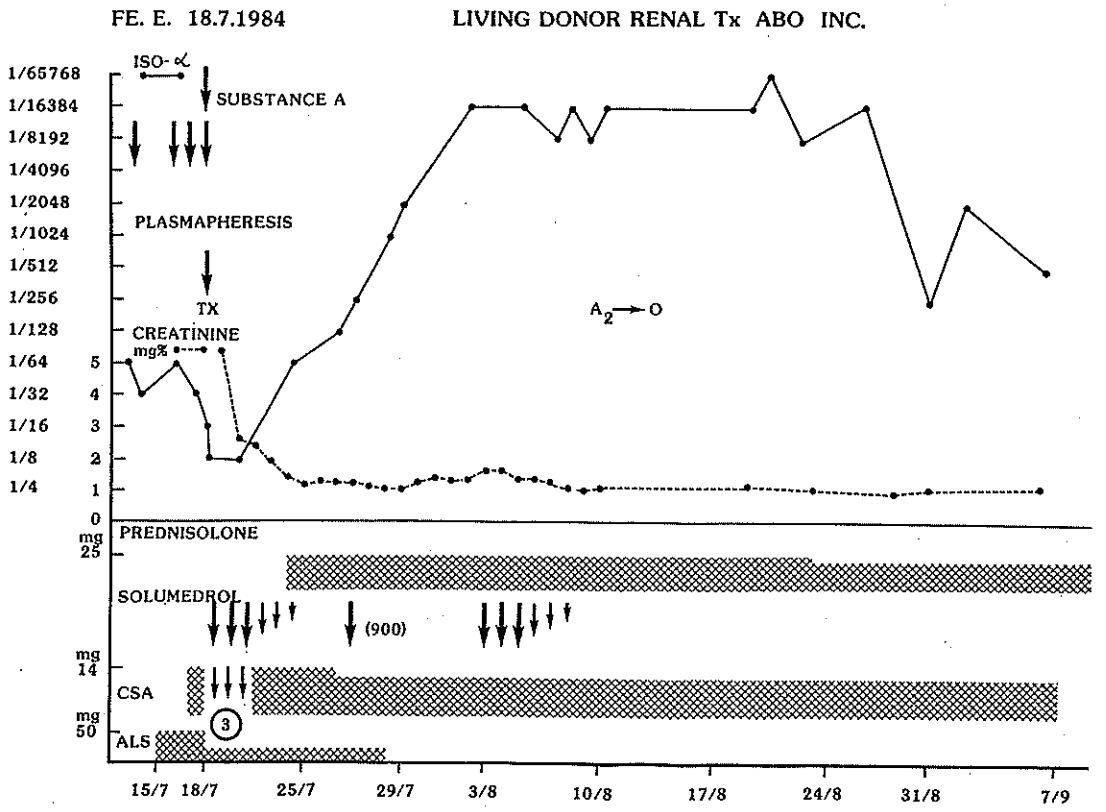

Fig. 1. — Two years follow-up of a human ABO-incompatible renal allograft with a normal renal function. The patient is maintaining a high isoantibody titer against the donor incompatible blood group.

regimen as a human recipient of an ABO-incompatible renal allograft could permit to breach the barrier presented by the PNA in the field of xenotransplantation.

In october 1984, the first pig (minipig) to baboon renal xenograft was thus performed in our laboratory. Preoperative plasmaphereses were performed under general anesthesia with ketamine (10 mg/kg) on day −2, −1 and on the day of transplantation to eliminate antipig xenoagglutinins; replacement therapy was achieved with a stable solution of human plasma proteins (SSPP). Soluble blood group substance A and B (Behasil Corporation, Miami) were administered to the recipient at the end of the last plasmapheresis in order to further eliminate remaining xenoagglutinins.

Under general anesthesia with ketamine, the animal was intubated and anesthesia was maintained with enflurane. Through a midline laparotomy, a classical splenectomy was performed; the distal portion of the aorta and vena cava were dissected free form adjacent tissues and circumvented. Both artery and vein of the kidney transplant, preserved in Euro-Collins solution, were anastomosed end-to-side to the recipient's aorta and vena cava with running 6-0 prolene sutures.

The ureter was then implanted into the bladder with a simple bivalve technique: the extremity of the ureter was splitted in two parts over a few millimeters, a small incision (2 to 3 mm wide) was made in the dome of the bladder and each part of the ureter extremity (each valve) was fixed with a 5-0 chro-

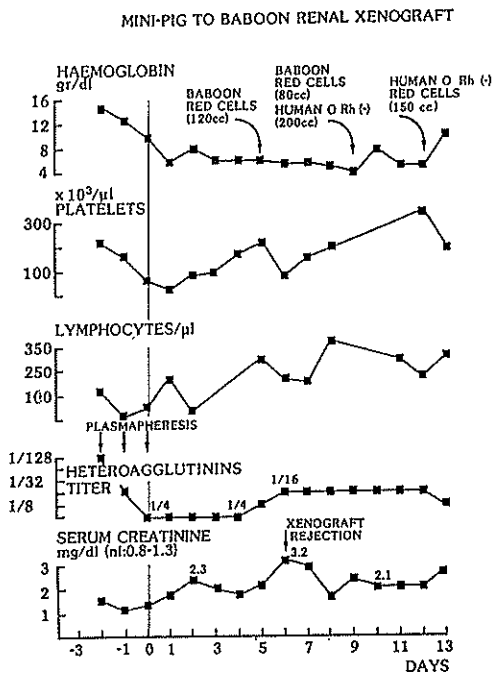

Fig. 2. — Minipig to baboon renal xenograft with maintenance of a satisfactory renal function up to the 6th postoperative day at which time a rejection crisis occurs, only partially reversible with antirejection therapy. Death from acute vascular rejection on the 13th postoperative day.

mic catgut to the bladder mucosa after having introduced the ureter in the bladder over a length of 1 cm. A bilateral nephrectomy was performed and the abdominal cavity was closed in two layers.

Recipient's immunosuppression was started on day −2 with rabbit antihuman antithymocyte serum (Fresenius, Germany) 18 mg/kg, given intravenously for ten days. Imuran was also administered IV daily, at a dose of 1 mg/kg while cyclosporine was administered intramuscularly at a dose of 10 mg/kg. Methylprednisone (10 mg/kg) was administered IV during operation and given intramuscularly daily thereafter at decreasing doses.

This renal xenograft succeeded in maintaining a satisfactory function for 13 days (fig. 2) whereas control grafts last a maximum of 35 minutes to a few hours. The combination of preoperative plasmapheresis with splenectomy and a quadruple immunosuppressive therapy thus demonstrated its effectiveness in prolonging a discordant renal xenograft far beyond the survival time that could be sofar obtained in this type of model.

In the series we have performed sofar, the follow-up of two other baboons is worth some more detailed description since they survived respectively 10 and 22 days. 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 was remarkably normal. There was no edema, practically no infiltrate; most glomeruli as well as the large vessels look normal. Only in a few glomeruli, some very small focal vascular thromboses could be identified.

The longest survivor of the series is a baboon who lived for 22 days (fig. 3). Renal function remained normal during the five first postoperative days at which time serum creatinine increased. A biopsy showed acute vascular rejection.

At the same time ciclosporine serum through level was more than 500 ng/ml, demonstrating cyclosporine cytotoxicity. Rejection was treated by administration of methylprednisolone at a dose of 250 mg/day for 3 days; cyclosporine was stopped temporarily and renal function returned bach to normal up to the 17th postoperative day at which time progressive impairment of the renal function occurred. On the 22nd day, the animal became anuric and was sacrificed. Autopsy revealed clearcut vascular irreversible rejection.

Discussion

Sofar survival of renal discordant xenografts has been limited to a few hours. The 3 pig to baboon renal xenografts which survived for more than one week and particularly the 2 baboons who maintained a normal function of the xenograft during most of their follow-up open new perspectives for the study of organ xenografting [8].

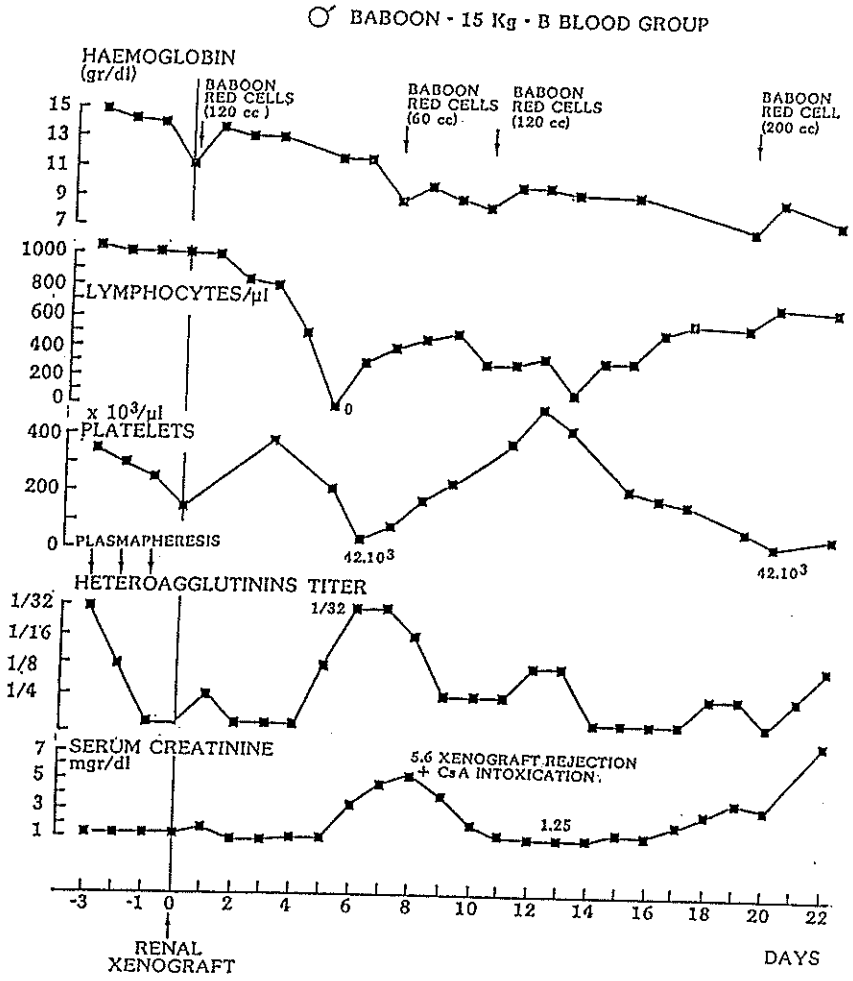

Fig. 3. — Minipig to baboon renal xenograft. Renal function remains normal up to the 6th postoperative day at which time a rejection crisis occurs. With antirejection therapy, renal function return to normal. The animal is sacrificed on day 22 as a result of an irreversible acute vascular rejection.

Although distinct major immunological differences exist between ABO-incompatible human renal allografts and minipig to baboon discordant xenografts, it is noteworthy that the immunological preparation and the immunosuppressive therapy applied in the human model are also effective in the xenograft model. In both models, recipients possess PNA against the donor, which produce vascular rejection when present at the time of transplantation.

ABO-incompatible human renal allografts and minipig to baboon discordant renal xenografts thus resemble to each other in the way they may be treated pre-, per- and post-operatively.

The pig to baboon model appears very well appropriate for research in the field of xenotransplantation. In other models, such as pig to dog or hamster to rat, the xenografts behave differently and it is not at all certain that the observations made in these models might be extrapolated to man.

In our model, there are however clear practical difficulties such as the need for a general anesthesia every time blood must be drawn

and frequent general anesthesia might also influence the recipient immunological reaction to the xenograft.

However, we may hope that the similarities observed between ABO-incompatible human renal allografts and minipig to baboon renal xenografts are suggestive of a similar immunological phenomenon in both models.

In our series of ABO-incompatible human renal allografts, some patients have a normal kidney function for more than 8 years while the isoagglutinin titer against the donor incompatible ABO blood group antigen is well above the preoperative level (fig. 1).

Some type of "accomodation" must take place, the mechanism of which remains a mystery.

The possibility that such an accomodation might exist also in xenografts opens a new frontier and gives hope for future clinical applicability of pig organ xenografts in man.

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