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Plasmapheresis and splenectomy in experimental renal xenotransplantation

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The major obstacle to xenografting between discordant species is the presence of natural preformed antibodies of the recipient species against the donor species producing acute vascular irreversible rejection of the transplanted organ. 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.

There exists a clinical model in human renal transplantation in which natural preformed antibodies may also produce acute vascular rejection of the transplanted organ: that is the transplantation of a kidney between ABO-incompatible donor and recipient. In both cases, ABO-incompatible human renal allografts and in discordant xenografts, natural preformed antibodies constitute the primum movens of the rejection phenomenon. Up to recent years, ABO-incompatibility was considered, and is still recognized by most transplant centers, as an absolute contraindication for renal transplantation.

Yet, we have demonstrated that long term successful ABO-incompatible kidney transplants may be obtained [1, 2, 4, 5] in the human by eliminating natural preformed isoagglutinins with plasmaphereses combined with splenectomy of the recipient. We have hypothesized that discordant kidney xenografts from pigs to baboons could also be successful by applying a similar treatment to the recipient animal.

The preparation, surgical operation and immunosuppression utilized in the experimental model are therefore based on our experience with human kidney transplantation from ABO-incompatible living donors.

Material and Methods

Minipigs were chosen as the donor species and baboons (*Hamadryas* and *Anubis*) were chosen as the recipient species. Five experiments have been performed.

Donor operation

A left nephrectomy was performed through a midline laparotomy and under general anesthesia. The kidney was very carefully dissected from the perirenal fat; both artery and vein were dissected from adjacent tissues; the ureter was easily dissected over a length of approximately 5 to 7 cm. The aorta proximally and distally to the origin of the left renal artery was freed and circumvented. The proximal aorta was clamped and the kidney was perfused with cold Euro-Collins solution through the distal aorta. The donor was sacrificed. The aorta was divided so as to keep a patch surrounding the origin of the renal artery. The kidney was then wrapped in a sterile bag with ice and put in the refrigerator.

Recipient operation

Preoperative plasmaphereses were performed under general anesthesia with ketamine (10 mg/kg) on day -2, -1 and on the day of transplantation to eliminate anti-pig heteroagglutinins; 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 to baboons #1 and #3 in order to further eliminate remaining heteroagglutinins. Under general anesthesia with ketamine the animal was intubated and the 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 from adjacent tissues and circumvented. The kidney transplant was then taken out of the refrigerator, out of the Euro-Collins solution and both artery and vein 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 end of the ureter was split open 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 chromic 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 anti-human anti-thymocyte serum (Fresenius, FRG) 18 mg/kg, given intravenously for 10 days. Imuran was also administered i.v. daily, at a dose of 1 mg/kg while cyclosporin was administered intramuscularly at a dose of 10 mg/kg. Methylprednisolone (10 mg/kg) was administered i.v. during operation and given intramuscularly daily thereafter at decreasing doses. Blood samples were taken daily under general anesthesia with ketamine (10 mg/kg i.m.).

Results

The baboons survived 1, 1, 10, 13 and 22 days. One animal was sacrificed on the day after operation: he was vomiting and serum creatinine was 5.3 mg%. Autopsy revealed a gastric dilatation but the renal xenograft appeared macroscopically normal. Microscopically, the histology was practically normal. A second baboon was sacrificed on the day after operation for anuria. Macroscopically and microscopically the transplanted kidney presented all the signs typical of an acute vascular rejection. In this animal it is important to note that it was not possible to lower the preoperative xenoantibody (agglutinin) titers below 1/32. Three baboons survived with satisfactory renal function. In the first of these three animals, the xenograft survived 13 days while serum creatinine was maintained between 2 and 3.5 mg% (Fig. 1). On the 6th postoperative day an increase of the serum creatinine was interpreted as being a sign of rejection; anti-rejection therapy was started with methylprednisolone and serum creatinine returned to prerejection level. No biopsy of the transplant was taken. On

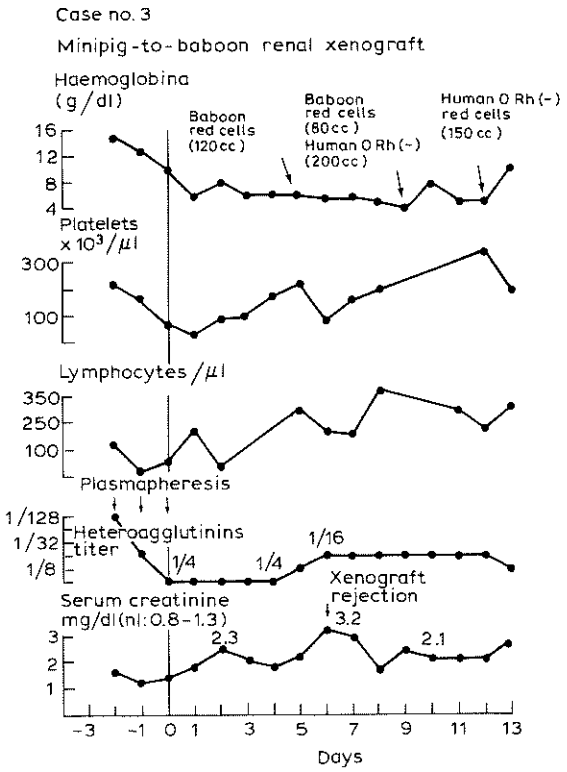

Fig. 1. Minipig-to-baboon renal xenograft. Renal function remained satisfactory up to the 6th postoperative day at which time rejection occurred which was partially reversible. The animal was sacrificed on the 13th postoperative day for acute irreversible rejection.

the 13th postoperative day, irreversible vascular rejection occurred and the animal was sacrificed. This rejection followed a second transfusion of human red blood cells which we were forced to administer because of the animal's anemia and the absence of compatible baboon blood.

The two other animals who survived 10 and 23 days, respectively, maintained normal xenograft function during most of their follow up. In baboon #4 (Fig. 2), serum creatinine reached 2.6 mg% on the first postoperative day but decreased rapidly to normal and remained within normal limits till the death of the animal from pneumonia on the 10th postoperative day. Heteroagglutinins remained low during the entire postoperative course while platelets remained high.

Baboon #5 (Fig. 3) was the longest survivor of this series. Renal function remained normal during the first five postoperative days at which time serum creatinine increased. A needle biopsy showed acute vascular rejection. At the same time cyclosporine serum through level was greater than 500 ng/ml, demonstrating cyclosporine

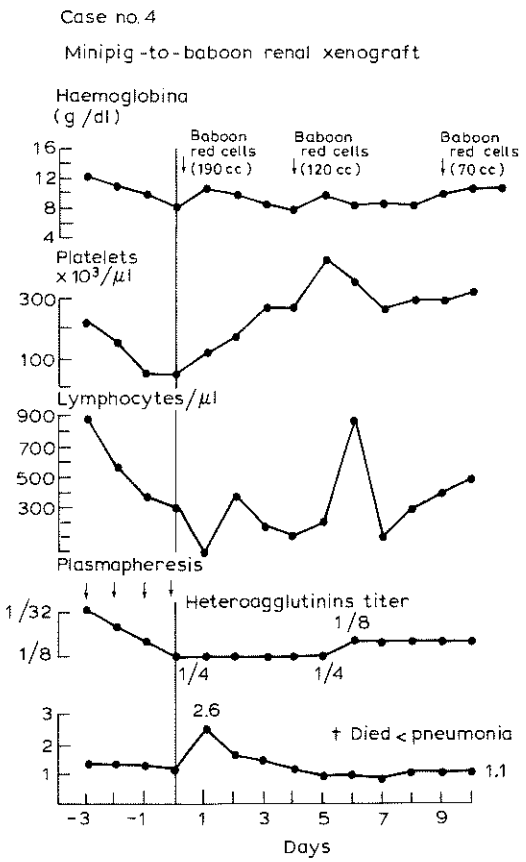

Fig. 2. Minipig-to-baboon renal xenograft. Renal function remained normal during the entire follow up. No rejection crisis. The animal died on the 10th postoperative day from pneumonia.

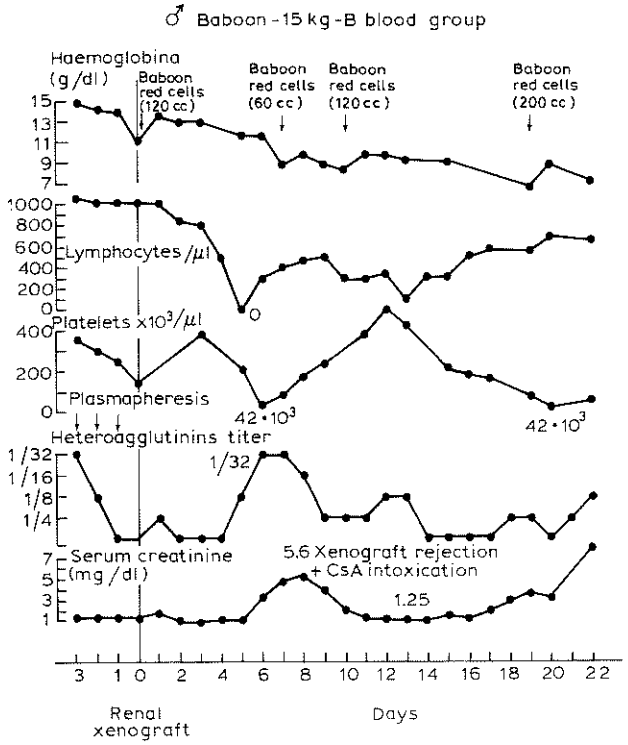

Fig. 3. Minipig-to-baboon renal xenograft. Normal renal function up to the 6th postoperative day at which time a rejection crisis occurred. Complete reversal of the rejection crisis. Occurrence of a second irreversible rejection and sacrifice of the animal on the 22nd day.

nephrotoxicity. Rejection was treated by administration of methylprednisolone at a dose of 250 mg/day for 3 days; cyclosporine was stopped temporarily, and renal function became normalized up to the 17th postoperative day at which time progressive impairment of the renal function began. On the 22nd day, the animal became anuric and was killed. Autopsy revealed apparent vascular irreversible rejection.

Discussion

The minipig-to-baboon renal xenograft model is clearly a discordant model. Survival of renal discordant xenografts till the present has been limited to a few hours. To our knowledge, this is the first report of normal function in discordant renal xenografts for a prolonged period of days. The three animals who survived for more than 1 week and particularly the two baboons who maintained normal function of the xenograft for longer than 2 weeks give us some hope for the development of organ xenotransplantation in man.

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 along with the immunosuppressive therapy applied in the human model are also successful in this xenograft model. Both models share the property of possessing natural preformed antibodies against the donor, which produce vascular rejection when present at the time of transplantation.

Recipient isoagglutinins against the donor incompatible blood group antigen(s) often increase at the time of a rejection crisis in human ABO-incompatible kidney transplantation [3]. Similarly, an increase of the heteroagglutinin titer was observed in cases #3 and #5 at the time of the rejection crises they both developed on the 6th postoperative day. In both rejection crises, there was a reduction in platelet counts, an observation which may be seen in some acute rejection crises of ABO-incompatible human renal allografts and correlates with microarterial thromboses of vascular rejection.

ABO-incompatible human renal allografts and minipig-to-baboon discordant renal xenografts thus immunologically resemble each other. They are similar in the way they are treated pre-, per- and postoperatively and in the way they behave at the time of a rejection crisis. The resemblance goes even further since in both cases the histological picture of rejection is typically that of a vascular rejection which may be reversible.

The reversibility of the rejection process came to us as the most promising feature of these experiments. To our knowledge, it is also the first time that reversibility of a rejection crisis of a renal xenograft is observed in a discordant model. From our experience in ABO-incompatible human renal allografts we already knew that acute vascular rejection crises may be reversible with appropriate therapy but reversibility generally requires intensive immunosuppressive therapy such as anti-thymocyte globulins or OKT3. In both cases of acute vascular rejection, in baboons #3 and #5, reversibility of the rejection phenomenon was obtained simply with supplementary doses of methylprednisolone.

Baboon #4 did not present any rejection crisis. This is also noteworthy. At all times during the postoperative period the heteroagglutinins remained low and the platelet counts remained high, two parameters which seem to correlate with good tolerance of the xenograft. Actually, at the time of death from pulmonary infection, histology of the xenograft was practically normal, which confirms the good acceptance of the graft.

The results of these experiments give hope for further progress in xenografting. Research in this field, however, will be difficult due to the lack of an appropriate model. It is not at all certain that the observations made may be extrapolated to man. In different models, such as pig-to-dog or hamster-to-rat, the behaviour of xenografts might be different and depend on the direction in which the xenografting is done, i.e., from which donor species the organ or tissue originates and which is the recipient species.

In our model, there are several practical difficulties such as the need for general anesthesia for the procedure and the need for intravenous replacement therapy. Because of the difficulty in monitoring the graft and the recipient during the postoperative period, the resulting treatment of both the recipient and the graft is obviously far from optimal. Frequent general anesthesia might also influence recipient immunological reaction to the xenograft. It is therefore even more difficult to extrapolate what the results could be in the human transplanted with pig kidneys.

We may hope however that the similarities observed between ABO-incompatible human renal allografts and minipig-to-baboon renal xenografts are suggestive of similar immunological phenomena in both models. If this hypothesis holds true, long term renal xenografts may be foreseen in a not too distant future.

Indeed, it has now become well known that it is possible to maintain long-term normal function of ABO-incompatible renal allografts even in the presence of a relatively high titer of isoagglutinins in the recipient serum. In our series of ABO-incompatible human renal allografts, some patients now have normal kidney function for more than 6 years while the isoagglutinin titer against the donor incompatible ABO blood group antigen is well above the preoperative level. The reason why these isoantibodies remain inactive on the specific antigen sites present on the graft endothelium is unknown. Some type of masking phenomenon must take place. But even if the mechanism of this phenomenon remains a mystery, the sole idea that organ grafts may function normally in this situation opens a new frontier for explanation and allows for sound optimism for the future of xenografts.

Summary

Five minipig-to-baboon renal xenografts have been performed using a protocol similar to that utilized in human ABO-incompatible renal allografts. The animals survived 1, 1, 10, 13 and 22 days. Satisfactory renal function was obtained in three animals. One baboon never presented any sign of rejection and had a normal function of the xenograft at the time of death from pneumonia on the 10th postoperative day. Two baboons, who survived respectively 13 and 22 days both presented a reversible rejection crisis on the 6th postoperative day. These results give hope for future applicability of xenografts in man.

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