Transplanting Organs from Pigs Transgenic for a Single Human Complement Regulatory Protein

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Considerable progress has been made over the past few years to achieve prolonged survival of pig hearts and kidneys which are transgenic for a single human regulator of complement activation, decay accelerating factor (hDAF), when transplanted into primates.

Heart

After heterotopic (i.e., not life-supporting) transplantation of nine hDAF transgenic pig hearts into the abdomen of juvenile baboons a maximum survival of 99 days (median 26 days) was achieved.[1] The immunosuppressive regimen consisted of cyclosporin A, steroids and mycophenolate mofetil, in addition to an induction course of cyclophosphamide until p.o. day 4. Two animals were sacrificed due to infection on p.o. days 10 and 15, respectively. The 99 day survivor was euthanased due to generalized weakness with a beating xenograft. Histology revealed an abdominal lymphoma. The remaining recipients were in good physical condition when euthanased due to the cessation of cardiac impulse of the transplanted xenograft. Acute vascular rejection (AVR) was documented in 2 recipients, in whom rejection occurred on days 12 and 15. Four out of the five longest survivors in the transgenic group (days 26, 32, 37, 44) also showed histological signs of rejection, although this was distinct in character from the AVR. There were small foci of necrosis and myocyte loss, together with microthrombi. Immunohistochemistry revealed less immunoglobulin and complement deposition in these hearts when compared to the hearts which underwent AVR. There was also evidence of a cellular infiltrate ($CD4^+/CD68^+$) in the areas of damage, phenotypically in keeping with macrophages. A small number of cells were detected which expressed CD2/CD8. These cells appeared later than the macrophages and were detectable in epicardial tissues and within the myocardium from day 15. As baboon natural killer cells do not stain for CD56, CD57 and CD122 the subpopulation of cells bearing a $CD2^+/CD8^+$ phenotype may however be NK cells. The xenograft from the day 99 survivor demonstrated some cellular infiltrate, but also showed evidence of intimal thickening of the myocardial vessels, consistent with chronic rejection.

Thus in pig to baboon xenotransplantation antibody and complement appear to be the most important factors of rejection in the early phase, later on cellular factors particularly macrophages and NK cells may also have a role.[2]

After orthotopic transplantation of six hDAF transgenic pig hearts into baboons immunosuppressed with the same protocol as in the heterotopic model maximum survival was 39 days with a median of 14 days.[3,4] The recipient with the highest levels of preformed anti-pig-antibody levels rejected the graft hyperacutely. Two baboons were sacrificed on p.o. days 2 and 11 for a pneumothorax and a sternal wound infection, respectively. Both grafts showed histological signs of mild acute vascular rejection. The remaining recipients died after 12, 22 and 39 days because of short-lived cardiovascular decompensations. All three demonstrated evidence of mild to moderate AVR with damaged myocardium varying in extent and severity. The pattern included fibrin and platelet thrombi within epicardial and intramyocardial vessels. The ischemic damage exhibited differential degrees of maturity consistent with a sequence of small vessel occlusions.

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The results demonstrate that there is clearly a need to better understand the mechanisms and effects of endothelial cell activation with subsequent expression of procoagulant factors and vessel thrombosis. Immunosuppressive strategies need to be developed which specifically address the role of macrophages and NK cells in delayed xenograft rejection. Further approaches to genetically engineer less antigenic donor pigs are likely to become a prerequisite for successful clinical heart xenotransplantation in the future.

Kidney

Several series of life-supporting kidney transplants of hDAF transgenic porcine kidneys into cynomolgus monkeys were performed which included a bilateral nephrectomy of the native kidneys.[5-10] A maximum survival of 35 days (median 13 days, n=7) was achieved using a triple immunosuppressive therapy with cyclophosphamide, cyclosporine A and steroids.[5] The four longest surviving recipients were sacrificed because of anemia. In a second series an additional splenectomy was performed and recombinant human erythropoietin was given to maintain sufficient hemoglobin levels.[6] In this group a maximum survival of 78 days (median 39 days) was observed, four of nine animals survived more than 50 days.[10] Most biochemical parameters measured in this study remained within normal ranges for several weeks. There was a tendency for the phosphate to fall initially and then calcium to rise, but without clinical sequelae.[11] Two animals demonstrated signs of post-transplant lymphoproliferative disease (PTLD) on p.m. examination.

In the following studies cyclophosphamide therapy was reduced to a short induction course until p.o. day 4, maintenance immunosuppression was achieved by cyclosporine A, mycophenolate mofetil (MMF) and steroids. In this study a maximum survival of 53 days (median 43 days, n = 5) was observed.[7] Splenectomy was not performed in a subgroup of six animals, consequently maximum was reduced to 35 days (median 15 days) without any obvious influence on graft rejection. However, significantly lower hemoglobin levels could be measured despite concomitant erythropoietin therapy.

In the two subsequent studies splenectomy was performed in all cases. When MMF was replaced by another mycophenolic acid sodium, ERL080, maximum survival was 51 days (median 29 days).[8] Acute vascular rejection was diagnosed in seven of eight grafts, six showed additional tubulitits with $CD8^+$ lymphocytic infiltrations. One animal had signs of PTLD on p.m. examination of the gut.

In the last study using SDZ RAD (a rapamycin analogue) instead of mycophenolate maximum survival increased to 71 days (median 33 days) with good renal function and a reduced incidence of rejection episodes, however, three out of seven animals had evidence of abdominal PTLD.[9]

Taken together the results demonstrate that hDAF-transgenic porcine kidneys are indeed able to maintain normal fluid, acid-base and biochemical homeostasis in primates. However, acute vascular and cellular graft rejection remain a hitherto uncontrolled obstacle in all studies despite fairly substantial immunosuppression as evidenced by the development of PTLD. The calcium-phosphate/vitamin D-parathormone interaction in this experimental model warrants further investigation. The development of anemia which can be prevented by splenectomy and recombinant human erythropoietin is not yet fully understood. Clinical studies appear to be premature before delayed xenograft rejection can be diagnosed and treated successfully.

. . . there is clearly a need to better understand the mechanisms and effects of endothelial cell activation with subsequent expression of procoagulant factors and vessel thrombosis.

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