Renal heterotransplantation from baboon to man: experience with 6 cases*

T E Starzl, T L Marchioro, G N Peters, C H Kirkpatrick, W E C Wilson, K A Porter, D Rifkind, D A Ogden, C R Hitchcock, W R Waddell

Departments of Surgery and Medicine, University of Colorado School of Medicine and Denver Veterans Administration Hospital, Denver; Department of Pathology, St. Mary's Hospital Medical School, London; and Department of Surgery, Hennepin County General Hospital, Minneapolis

SUMMARY

Six patients with terminal uremia due to glomerulonephritis or pyelonephritis were treated with heterografts from East African baboons. Immunosuppressive therapy was provided both before and after operation with azathioprine and prednisone and postoperatively local transplant irradiation and actinomycin C were administered intermittently. The individual rejection episodes in the post-transplant period could be reversed relatively easily but these recurred vigorously and repetitively, making it impossible to relax the stringent requirements of antirejection therapy. The continued need for high-dose immunosuppressive therapy precipitated lethal infections in the majority of cases.

The patients lived for 19 to 98 days after heterotransplantation. Four died with the baboon kidneys still in place after 19, 23, 35, and 49 days. In the other two cases the heterografts were removed after 60 and 49 days respectively, at a time when urine excretion was still present, and homografts from volunteer convict donors were placed on the opposite side. Both the latter recipients died of septic complications following the second operation, after 39, and 44 days. Complete cessation of heterograft urine excretion appeared only in two cases, although renal function was failing in the remainder prior to death or before removal of the heterografts. The relation of the renal function to changes in the heteroagglutinin and hemagglutinin titers is described.

After residence in the host for 19 to 60 days, all the heterotransplants were heavily infiltrated with plasma cells and large lymphoid cells with pyroninophilic cytoplasm. There was also disruption of peritubular capillaries, interstitial edema, widespread tubular damage, swelling of endothelial cells lining arterioles, fibrinoid necrosis of the walls of arterioles and interlobular arteries by fibrin and platelet deposits on the

*Reproduced, with permission, from *Transplantation* (1964) 2, 752–776

▶ Although we predicted from the Reemtsma/Hitchcock experience that the baboon kidneys would not be hyperacutely rejected, the specter of more subtle antibody rejection hung heavily over the trials, prospectively and afterwards. It would be another year before hyperacute rejection associated with lymphocytotoxic antibodies was recognized in ABO-compatible allograft recipients[1]. Consequently, a study of such antigraft antibodies was not done. Instead, attention was focused by David Talmage's immunology fellows (Charles Kirkpatrick and W E C Wilson) on the effect of hetero(xeno)specific hemagglutinins.

The baboon xenografts functioned for a mean of 41 days when there was ABO compatibility (n = 3) versus 21 days when there was not (n = 3). Although this suggested an important ABO effect, there was serologic evidence that xenospecific hemagglutinins also bound to the transplanted kidneys in every case. It appeared from the clinical observations, and especially from Porter's histopathologic analyses, that humoral xenograft rejection was uncontrollable with cell-directed immune suppression, even in the phylogenetically close baboon-to-human combination.

Therefore, we concluded that, "until improved methods of management become available, further trials do not seem justified, and none are contemplated by us until that time". The moratorium lasted more than 28 years until our two laboratory-based attempts at baboon-to-human liver transplantation of 1992–93. These also failed because of a less obvious humoral rejection, but with unmistakable complement activation despite complete freedom from cellular rejection out to 70 days. As in 1964, the rejection of 'concordant' xenografts was a 'slow motion' version of that seen with 'discordant' xenografts[2]. It was time for a new moratorium.

The humoral component of ▶

intima. The pre-glomerular vascular lesions were accompanied by focal infarcts and extensive interstitial hemorrhages. All the pathologic changes were more severe then those seen by Reemtsma in comparable series of chimpanzee-to-man heterotransplants, where cellular infiltration was slight and vascular lesions uncommon in the presence of major blood group incompatibility between donor and recipient.

During the developmental era of vascular surgery, five clinical renal heterotransplantations are known to have been tried, each with a different type of animal donor (4,7,16,19). Significant renal function was not obtained in any instance, and the longest survival was 9 days. No additional attempts at heterotransplantation were made in the ensuing 40 years, and the tacit assumption became firmly entrenched that such avenues of investigation presented insurmountable biologic difficulties.

In 1963, Reemtsma (12,14) and Hitchcock (2) and their associates re-examined the possibility that heterograft function could be obtained and sustained with the aid of various immunosuppressive agents. It was established that immediate urine excretion of chimpanzee (12–14), rhesus monkey (12), and baboon kidneys (2) followed after transplantation to the human, and that maintenance of relatively protracted chimpanzee heterograft function could be expected at least in the occasional case.

The present study is an account of a clinical study of renal heterotransplantation carried out at the University of Colorado Medical Center in December, 1963, and January, 1964, using baboons for donors. By comparison of the results with those previously obtained with homotransplantation (17) it was hoped to define the differences and similarities of homograft and heterograft behavior in the human host. In addition, it became possible as the result of an exchange of functional and pathologic data with Reemtsma to arrive at tentative conclusions concerning the biologic suitability for human heterograft donation of different subhuman primates.

Results

Clinical course. Four patients died with baboon kidneys still in place [?]. In Cases 3 and 4, the heterografts were removed after 60 and 49 days respectively, and homografts from volunteer convict donors were placed on opposite side. Survival after the second operation was 39 days in Patient 3 and 44 days in Patient 4. Complete cessation of heterograft excretion occurred only in Cases 2 and 5 (Table 3 [not shown]), although renal function was failing in the remainder prior to death (Patients 1 and 6) or before removal of the transplant (Patients 3 and 4). All patients exhibited a marked early clinical improvement at the time of initial diuresis and for varying periods thereafter. Recovery was, however, interrupted in each instance except Patient 1 by early rejection crises, which were characterized by transplant site tenderness, and by multifaceted evidence of acute renal failure. The timing of the rejection episodes is indicated in Table 4 [not shown] and the influence upon renal function is graphically portrayed in Figures 2–4 [not shown].

Ultimately, each of the last five cases became unmanageable because of the repetitive and closely-spaced rejections. Although the individual crises could be at least partially controlled in most instances, with local transplant irradiation, actinomycin C or increases in steroid dosage, the adverse consequences could not be completely reversed before the onset of the next assault. The cumulative effect was progressive deterioration, interrupted by incomplete remissions. In Patients 3 and 4, removal of the heterografts was precipitated by the sudden formation of masses in the transplant areas, of such magnitude in Case 3 as to produce massive edema of the right leg which was apparently due to local compression of

---

▶ xenograft rejection was discussed in our 1964 article as an Arthus reaction, the same term (or alternatively 'Shwartzman reaction') used to described hyperacute allograft rejection in patients who had preformed antigraft cytotoxic antibodies (classical pathway of complement activation) but also in exceptional patients who were antibody free (alternative pathway)[3]. Thus, it was recognized almost from the beginning that humoral rejection of allografts under specific circumstances and that of xenografts involved an acute inflammatory reaction that would not yield to conventional immune suppression.

John Najarian, in commenting 30 years later on his own classical studies[4], has pointed out how little progress has resulted from the cyclic 'rediscovery' of the pathogenesis of xenograft rejection, and of treatment strategies, using increasingly sophisticated technologies to delineate what was already quite obvious by the late 1960s. An exception to the futility was the recognition by Gus Dalmasso and Fritz Bach that the seminal problem of complement activation could be resolved by providing the target donor tissues with complement regulatory peptides of the recipient species. This objective was achieved when 'humanized' transgenic animals were produced by David White in England and by John Logan in the USA with the collaboration of Jeffrey Platt and other colleagues. With this progress in cracking the shell of the xenotransplant problem, what remains to be done is the induction of organ acceptance by the same chimerism-dependent mechanisms that have spawned successful allotransplantation[5].

References

1 Terasaki, P I, Marchioro, T L and Starzl, T E (1965) in *Histocompatibility Testing*, pp. 83-96, National Academy of Science/National Research Council, Washington, DC
2 Starzl, T E *et al.* (1994) *Immunol Rev* 141, 213–244
3 Starzl, T E *et al.* (1968) *New Engl J Med* 278, 642–648
4 Najarian. J S (1996) *Xeno* 4, 34–35
5 Starzl, T E *Immunol Today* (in press)

the venous and lymphatic systems. At the same time of extirpation, the swollen and boggy heterografts were surrounded with 500 to 1000 cc serosanguineous fluid under considerable pressure.

Because of the intensity and perseverance of rejection, it was not possible to successfully relax the magnitude of immunosuppressive measures. Every attempt to reduce prednisone was followed by serious rejection. The prolonged use of doses in excess of 100 mg/day resulted in profound hypercorticism. Three of the six patients developed steroid diabetes and one required treatment with insulin. The extraordinary degree of immunosuppression required to maintain even mediocre renal function undoubtedly contributed to the septic complications which played a role in the unfavorable outcome of all but Patient 5. In Cases 3 and 4, in which homografts were ultimately used to replace the excised baboon kidneys, the protracted initial course of stringent antirejection therapy appeared to have jeopardized the prognosis for the same reason, since sepsis was a terminal event in both patients. Patients 1 and 2 had early pulmonary emboli with infarction and abscess formation. Resection of the lower lobe and lingula was performed in Case 2 in an attempt to eliminate this septic focus. The sources of venous anastomoses. Those patients receiving heterografts from baboons with compatible blood types had more sustained function than those which did not. (Table 2) [not shown].

The alternations in renal function (Fig. 2–4) were similar to those which characterize homograft repudiation (17). Urine volume fell. Sudden rises in BUN and serum creatinine occurred with declines in creatinine clearance. The partial reversibility of these changes was encouraging. In many instances (Table 4), this was accomplished with the combined use of actinomycin C and local irradiation. The efficiency of each of these agents alone was also demonstrated on other occasions (Table 4), the most clear-cut benefit being with local x-ray therapy (Figs 2, 4). In Cases 4 and 6, secondary diuresis occurred with volumes as high as 7870 and 4750 ml (Fig. 3, 4) [not shown]. The relative ease with which partial remission was achieved was misleading. Functional restoration was either short-lived or incomplete. The shortest interval between the first and subsequent rejection crisis was 3 days; the longest was 45 days (Fig. 2). Between these dramatic occurrences, there was frequently a subtle diminution in function (Figs 2–4). Patients 2 and 5 ultimately became anuric. The last creatinine clearance measured in Cases 1, 3, 4 and 6 before removal of the heterografts or death of the patients were 59.8, 16.1, 25, and 14.8 ml/min.

In addition to the other features of rejection described above, a consistent alteration of urinary composition was observed. A sharp decrease in urinary sodium and chloride concentration accompanied the relative oliguria of the rejection episode (Figs 2, 3) in every instance (Table 7 [not shown]). A concomitant increase in urinary urea nitrogen concentration was usually observed. With reversal of rejection, the diminished sodium excretion tended to return to the previous level at the same time as improvement occurred in urine volume, creatinine clearance, and BUN. That none of these alterations is a unique feature of heterografts was evident from the fact that similar changes were observed in Case 4 during a single rejection after placement of a homograft.

Infectious Complications. A variety of infections were observed before and after heterotransplantation (Table 8 [not shown]). Those occurring after institution of immunosuppression was started were effectively treated. Those occurring after institution of immunosuppression were not affected by antibiotic therapy, the infectious complication contributing to 5 of the 6 deaths (Table 8). It had been pointed out previously (15) that several consequences of immunosuppressive therapy summate to render the patient abnormally susceptible to infectious complications. These effects were apparently magnified in the heterotransplantation cases in which maximal suppressive therapy was required for even temporary survival of the grafted organ. The extreme degree of immunosuppression was reflected by the presence of significant leukopenia (3000/mm^3 or less) in three patients, hypogammaglobulinemia (600 mg% or less) in two, and glycosuria in three. It is probably for these reasons that only one of the six known bacterial infections occurring after the institution of immunosuppression was controlled. Antibiotic therapy may have contributed to development of systemic fungal infections.

Gross Pathologic Studies. The baboon kidneys were all enlarged, with a mean weight of 108 gm. This increase was greatest (180 gm) in Case 2, where function ceased at 25 days following an irreversible phase, and least (65 gm) in Case 1, where the patient had never experienced a clinically recognizable rejection episode and where the creatinine clearance was still 59.8 ml/min hourly before the patient died from a pulmonary embolus.

The capsules were a little thickened and stripped easily. In Case 1 the subcapsular surfaces were smooth, light brown and speckled with petechial hemorrhages; when cut, the kidneys bulged slightly and there was some blurring of the corticomedullary junction. The kidneys from Case 5 were a uniform reddish purple and it was not possible on the cut surfaces to differentiate between cortex and medulla. However, the other four pairs of transplants were mottled with irregular blotchy hemorrhages, measuring up to 0.5 cm in diameter, and paler yellowish infarcted areas which were each surrounded by a bright red zone several millimeters wide (Fig. 12 [not shown]). There were also scattered petechial hemorrhages. The cut surfaces of these kidneys bulged and similar hemorrhagic and paler areas were seen in the cortex and extending in the cortex and extending into the deep red medulla. The main renal artery and vein were patent in all cases, although the blind pouch of baboon aorta was filled with laminated thrombus in Case 2. The right kidney from Patient 1 was surrounded by a large hematoma which might have caused some compression of the renal artery on that side. The ureters had swollen, often hemorrhagic walls, with narrowed by patent lumens. There were

petechiae in the calyces and renal pelves.

Microscopic Pathologic Studies. All the transplanted kidneys were heavily infiltrated by cells (Figs 13, 14 [not shown]). This was most pronounced in Cases 1 and 3 and least obvious in Case 6. The cells, which were found predominantly in the cortex, were distributed in a patchy fashion in four of the pairs of transplants, but in Patients 1 and 4 they were scattered diffusely throughout the interstitium. Many were plasma cells and some of these possessed two or even three nuclei. Others were cells with varying amounts of pyronin-positive cytoplasm and large pale nuclei, with prominent nucleoli. In Case 1 occasional such cells were in mitosis. There were also a few small lymphocytes, and in Cases 1 and 3 eosinophils were frequent. Phagocytic cells which looked like lupus erythematosus (LE) cells, were present in small numbers in the interstitium of Cases 2 and 3. Some erythrophagocytosis was seen in the transplants from Patient 1. Interstitial edema was a feature of all these heterotransplants (Fig. 14). It was most severe in the heaviest kidneys which were those from Cases 2 and 3. Scattered focal interstitial hemorrhages were present in all of the heterotransplants and accounted for many of the red blotches seen grossly. Although they were infrequent and small in the kidneys from Case 1, they were widespread and large in Cases 3 and 4. Focal small infarcts, while present in all the transplants, were least apparent in patients 1, 3 and 4 where function terminally had been fair and whose last creatinine clearances ranged from 16.1 ml to 59.8 ml/min. Some of the infarcts were hemorrhagic (Fig. 15 [not shown]). Almost total infarction was present in Case 5. Peritubular capillary destruction was widespread in all the cases (Fig. 14). Surviving capillaries usually contained marginating pyroninophilic and lymphoid cells. Swelling of the endothelial cells lining the afferent arterioles was obvious in three of the pairs of kidneys.

In four of the transplants there was focal fibrinoid necrosis of the walls of the interlobular arteries and arterioles (Fig. 16 [not shown]). There may also have been similar changes in Case 5 but widespread infarction made interpretation of vessel changes impossible. Rupture of the internal elastic lamina of affected vessels was common. Case 1 showed no vasculonecrotic lesions. Fibrin and platelet deposits on the intima of interlobular arteries were seen in all the transplants and caused narrowing of variable number of the vessels. Blockage of some lumens was completed by superimposed thrombus. In Case 2 replacement of the fibrin-platelet deposits by fibroblasts had occurred with breakdown of the platelets to leave fat droplets in the deeper parts of the occluding layer. Obvious secondary thrombosis of large arteries and veins was present in the two pairs of heterotransplants which ceased functioning many days before death of the patient (Cases 2 and 5).

Tubular necrosis with evidence of regeneration was present in all the cases. This was most severe and widespread in Patients 2, 3 and 6. Casts of protein and cell debris were frequent. There was also some red cell and pigment casts. The glomeruli were relatively well preserved. There was some hypertrophy of the tufts and prominent granularity of the juxtaglomerular cells in the pair of transplants that functioned for 60 days (Case 3). Hyperplasia and increased granularity of the juxtaglomerular body was present in the one case (Patient 4) in which hypertension had been present during the postoperative course.

Discussion

The present study was designed to thoroughly test the feasibility of renal heterotransplantation from baboon to man, with the best therapeutic regimen currently available. This objective was met. There was no surgical mishaps. Each of the heterografts was inserted under ideal technical conditions. Although the transplanted tissue was not weighed at the time of operation, it was predicted from previous ratio determinations of renal weight/total body weight that the total renal mass ranged from 32 to 82 gm, half or less than that of a single human kidney.

The primary cause of failure in all but one case was inability to control rejection. When other nonrenal complications supervened, most were related to the need for continuous high-dose immunosuppressive measures. Generally severe pathologic changes were present in the heterotransplants. The cellular infiltration was heavier than that seen in any of the homografts from the University of Colorado renal homotransplantation series (9), which were removed or recovered at autopsy. In Cases 1 and 3 the infiltrate approached in severity that seen in canine homotransplants. The accompanying widespread damage to peritubular capillaries had resulted in extensive tubular necrosis. Arterial and arteriolar interstitial hemorrhages. Even the kidneys from Case 1, where no rejection episode was detected, showed heavy cellular infiltration, damage to peritubular capillaries and early patchy tubular necrosis. Under the circumstances of this study, chronic survival after baboon-to-man transplantation seems, therefore, to be a virtual impossibility. Unless improved methods of management become available, further trials do not seem justified and none are contemplated by is until that time.

The recent use of other subhuman primates allows some tentative conclusions concerning the relative biologic suitability of simian donors for human heterotransplantation. From a pathologic point of view, the vigor of the immunologic reaction has seemed to be much less with chimpanzee tissue, in contrast to baboon and rhesus monkey heterotransplants which evoke a fierce response on the part of the human host (9). In this connection, it is noteworthy that one patient treated by Reemtsma with paired chimpanzee heterografts has essentially normal renal function 22 weeks after operation.

It was pointed out earlier that baboon heterograft rejection was not overtly different in many respects from that commonly observed with homografts. The differences were quantitative in that repudiation of the alien tissue was more vigorous and insistent. Despite the similarities it is pertinent to consider that the rejection of heterografts may involve immunologic pathways different from those of homografts. Specifically, it might be expected that circulating free antibodies could play a dominant role. In

laboratory experiments with cross species transplantation, destruction of whole organ heterografts has been observed to occur with such rapidity (within minutes or hours) that only a humoral mechanism could provide a satisfactory explanation (1). The immediate consequences in these experiments were indistinguishable from those which have been observed in two human cases in which homografts were transplanted between donors and recipients of incompatible blood types (17), and in an A to O chimpanzee-to-human heterotransplantation performed by Reemtsma (14). Since all six patients in the present study had preformed heteroagglutinins directed against baboon erythrocytes, it is surprising that a similar immediate repudiation did not occur, particularly since measurable heteroagglutinins disappeared entirely from the peripheral blood at some time during the postoperative course in each case.

Except as a general index of immunologic activity, the significance of the changes in host heteroagglutinins, and their cyclic variation during rejection episodes, is not known. The disappearance of heteroagglutinins from the peripheral blood indicated that antigenic determinants specific for this serum antibody were present on the renal cells. The later rises in titer could be explained by an intensification of antibody formation accompanied by saturation of all available binding sites within the kidney. The possibility receives some support from the fact that the increases in heteroagglutinin titers tended to occur at the same time as the acute rejection episodes.

There are a number of possible explanations for the failure to observe immediate rejection in the presence of a preformed antibody directed against the heterograft. Stetson and Demopolous (18) have postulated that an intrarenal Arthus reaction is the effector pathway mediating humoral rejection. Since the intensity of the Arthus reaction is a function of the concentration of bivalent antibody (10), it is conceivable that the quantity of recipient heteroagglutinin was insufficient to saturate all the antigenic sites on the kidney cells. The total disappearance of the heteroagglutinins is evidence in favor of this. Finally, it is possible that the release of chemical effectors of rejection, perhaps because a final step of the reaction was blocked by immunosuppressive therapy.

It is impossible to be certain from present evidence if there is any fundamental difference in the mechanism of rejection in these heterografts as opposed to homografts. With heterografts, both humoral and cellular elements seem to play a role. With homografts, the principal emphasis in the past 2 decades has been directed to the concept of cell-mediated rejection, primarily because of the paucity of evidence implicating measurable serum factors. More recently the potential role of humoral component of homograft rejection has received increasing support from several lines of evidence (3,6,11,17). The differences between heterograft and homograft rejection may, therefore, be more apparent than real.

Another hemagglutination system was studied which has immediate practical importance since it influences donor selection. It was pointed out in the "methods" section that A and B antigens are not detectable in baboon erythrocytes for which reason the animals were blood typed with salivary tests and by the presence of reciprocal hemagglutinins (21). The alterations in specific anti-A and anti-B titers provide evidence that these antigens do not exist in renal tissue despite their absence on the red cells, a situation comparable to that described by Weiner and his associates (20) for the Rhesus monkey.

The marked changes in titer after heterotransplantation of AB blood type mismatches and the absence of this finding in the compatible cases is strong evidence of hemagglutinin binding by the heterograft. As with the heteroagglutinins, this did not prevent immediate function, which survival was not as long and the quality of renal function was a slightly lower level in the mismatched group. It would, therefore, be considered inadvisable in the future clinical investigations of heterotransplantation to accept such incompatible donor-recipient combinations.

[References not shown]