THE PATHOPHYSIOLOGY OF RENAL ALLOTRANSPLANTS IN SUBJECTS ON IMMUNOSUPPRESSIVE THERAPY*

SAMUEL L. KOUNTZ and ROY COHN

Department of Surgery, Stanford University School of Medicine, Palo Alto, Calif., U.S.A.

It is well established that the vascular system of a renal allograft is the primary site of the immunological reaction in both the unmodified and modified hosts (Kountz et al., 1963; Porter et al., 1964; Dempster et al., 1964). The reaction is initiated by host cells arriving in the graft via the bloodstream. These cells attack the vascular endothelial cells of the small intertubular blood vessels of the graft resulting in areas of cytoplasmic continuity. Progressive vascular destruction of these blood vessels occurs with ultimate destruction and blockage of the larger blood vessels. During the early phases of the rejection process and even up to the time of oliguria it has been demonstrated (Williams et al., 1964) that the glomeruli and tubules are spared from progressive anatomical damage.

Recently, Knudsen et al. (1967), using serial angiography, serial measurements of renal function, and light microscopy, more precisely localized the anatomical part of the intra-renal vascular system undergoing progressive destruction. They observed venous filling defects on angiography which on microscopic examination proved to be perivenous infiltration of host cells. It was after the appearance of this anatomical lesion that renal function began to decline.

Several investigators (Cohn and Kountz, 1964; Jackson and Mannick, 1964; Retik et al., 1967; Dibbell et al., 1966) have demonstrated a decline in renal functions, i.e. in total renal blood flow (RBF), effective renal plasma flow (ERPF), and the glomerular filtration rate (GFR) during rejection. The absence of glomerular and tubular damage until the time of oliguria, or early anuria, suggests that serial and simultaneous measurements of ERPF and GFR might be used to detect and monitor the immunological reaction in the modified as well as the unmodified hosts. This assumption is true if little ischemic damage occurs at the time of transplantation and the immunosuppressive therapy itself does not produce glomerular or tubular damage. The pathophysiological patterns might be expected to express individual host-graft histoincompatibility. To test this hypothesis, serial and simultaneous determinations of ERPF, GFR and serum creatinine were performed in subjects with allotransplants receiving immunosuppressive therapy.

MATERIALS AND METHODS

The following groups of subjects with renal allotransplants received from living donors were studied: (1) Dogs with autografts (5) and unmodified allografts (20); (2) Dogs (20) with allografts receiving oral azathioprine (Imuran) 2 mg/Kg and prednisone 1 mg/Kg daily until incipient rejection developed, then one oral dose of azathioprine 10 mg/Kg and methylprednisolone (Solu-medrol) 20 mg/Kg plus 0.5 mg actinomycin-D (Cosmegen) were given intra-

* Supported in part by U.S. Public Health Service Grant HE-02278-10, FR-5353-05, CRC grant, and Santa Clara County Heart Association grant. We thank Messrs. Cornelis Ploeg, Stephen Freese, Miss Linda Williams and Mrs. Margaret Wilson for technical assistance.
venously; (3) Dogs (25) with allografts receiving oral azathioprine 1 mg/Kg daily and methylprednisolone 20 mg/Kg and 0.25 actinomycin-D plus 100 mg heparin directly into the graft via a catheter in the renal artery in the first 48 to 72 hours. Subsequent intra-renal doses were administered at the time of incipient rejection; (4) Humans (10) with allografts receiving oral azathioprine 2-4 mg/Kg daily and prednisone 0.25 to 1 mg/Kg daily. At the time of incipient rejection one single dose of 10 mg/Kg azathioprine orally and 20 mg/Kg methylprednisolone and 0.5 mg actinomycin-D were given intravenously; (5) Humans (18) with allografts receiving oral azathioprine 1-2 mg/Kg and 0.25 to 0.5 mg/Kg prednisone daily. During the first 24 to 48 hours the grafts were locally infused with normal saline that contained 20 mg/Kg methylprednisolone, 0.5 mg actinomycin-D and 20 mg% heparin via a lambda pump.

The catheter was left in the transplanted renal artery for repeat doses at the time of incipient rejection. Between treatments of incipient rejection the catheters were kept patent by slowly infusing 20 mg% heparin and saline as earlier reported (Cohn and Kountz, 1967). Incipient rejection was determined when the effective renal plasma flow declined to 40-60% of normal (Kountz et al., 1965). The glomerular filtration rate (GFR) was estimated in most cases by creatinine clearances. In the dogs, and in some human cases, the clearance of technetium-99 m (V)-citrate complex was used (Kountz et al., 1967).

Fig. 1. Typical pathophysiological patterns in untreated allografts in dogs showing: (a) a rapid rejector, (b) a slower rejector and (c) an autotransplant.
RESULTS

Two pathophysiological patterns were observed in allografts in unmodified hosts: (1) simultaneous declines in ERPF and GRF (rapid rejectors 6 days or less, 40% of 20); and (2) sequential declines in ERPF and GFR (slower rejectors 6 days or more, 60% of 20) with autografts exhibiting stable ERPF and GFR (Fig. 1). To detect these changes, ERPF and GFR had to be measured daily as GFR would frequently lag behind ERPF by only 24 to 48 hours. In all cases elevation of the serum creatinine followed declines in ERPF and GFR. However, each graft exhibited a slightly different pathophysiological pattern believed to be characteristic of the specific graft-host combination.

In the modified hosts, two groups could also be distinguished from the pathophysiological expression of the first rejection at the dosage level of the immunosuppressive therapy used. In most instances there was a sequential decline in ERPF and GFR, but in about 20% of the 20 animals the first rejection occurred before 10 days with GFR declining very shortly after a decline in ERPF, less than 48 hours. In the 20 animals studied, 13 died of uncontrolled rejection and 7 from toxicity of immunosuppressive therapy with a mean survival of 53 days (range 27 to 196). In this series, the serum creatinine fell after a decline in ERPF and GFR. Frequently, there was a lag period after reversal of rejection for GFR and serum creatinine to return to normal while the response of ERPF was frequently quite rapid. Typical clinical courses are shown in dogs a and b in Fig. 2.

![Graph showing ERPF and GFR in dogs A, B, and C](image)

Fig. 2. Typical pathophysiological patterns in treated allograft in dogs showing specific host-graft patterns (a and b). Dog c received local treatment. Note that the first rejection is prolonged past 20 days.
In the animals receiving intra-renal immunosuppressive therapy, identical patterns of ERPF and GFR were noted (Fig. 2c). However, the first rejection was always prolonged and initial graft function was better with a more rapid response to treatment of a rejection crisis. In 25 dogs the mean survival was 84 days (range 39 to 178). A major problem was accidental removal of the catheter with uncontrolled rejection. The overall dosage of immunosuppressive therapy in this group was slightly lower. Local infusion of azathioprine in 4 dogs did not show the immediate hemodynamic responses at the time of incipient rejection which was characteristically observed when methylprednisolone was used.

In human subjects on oral and parenteral immunosuppressive therapy, three pathophysiological patterns were observed: (1) rapid rejectors (10 days or less) with simultaneous declines in ERPF and GFR (Fig. 3a); (2) slower rejectors with sequential declines in ERPF and GFR (Fig. 3b); and (3) stability of both ERPF and GFR (Fig. 3c). In all of these subjects the serum creatinine began to rise only after a decline in ERPF and GFR. In these 10 recipients, there was one technical failure, sister to brother, 3 unrelated failures with all showing early and simultaneous declines in ERPF and GFR. All of these patients died from drug toxicity in an effort to control severe rejection. One uncle to nephew allograft exhibited simultaneous declines in ERPF and GFR, and this recipient also died from infection secondary to drug toxicity in an effort to control severe rejection. There were no survivors with early rejection.

Fig. 3. Typical clinical courses of three patients representing the three pathophysiological patterns: (a) patient W.A. shows simultaneous decline in ERPF and GFR at the time of the first rejection; (b) patient R.B. shows sequential decline of ERPF and GFR at the time of the first rejection; (c) patient J.H. shows relative stability of ERPF and GFR.

338
exhibited by simultaneous declines in ERPF and GFR. In the other 5 patients, 4 showed sequential declines in ERPF and GFR with the first rejection which was easily controlled, and 1 showed stability of ERPF and GFR over a long period of time, now two years. All of these patients have normal renal function except one, now 3 years, who has mild azotemia.

In the 18 subjects who have been treated by the local intra-renal technique, one death has occurred which was due to infection secondary to drug toxicity. The other 17 patients are all well with normal renal function, 1 to 13 months. In this group, 10 showed sequential declines in ERPF and GFR at the time of the first rejection and 8 have had stability of ERPF and GFR. The function of these grafts immediately after transplantation was better than the above 9 successfully transplanted non-perfused grafts, and no early simultaneous declines in ERPF and GFR were seen. However, only one of the grafts came from an unrelated donor. The one death due to infection was in a mother to daughter transplant.

DISCUSSION

The data presented here indicate the value of serial ERPF and GFR measurements following renal allotransplantation as diagnostic and therapeutic tools. Interpretation of these measurements is more reliable if the graft is obtained from a living donor and little ischemic damage occurs at the time of transplantation. It is possible that the group showing early decline in ERPF and GFR received grafts that suffered tubular damage during transplantation, but all kidneys came from living donors and exhibited 2 to 4 days of good function. The early decline of ERPF during rejection allows increased therapy to be given before the reaction has resulted in glomerular or tubular damage from ischemia. The rapid upward response of ERPF to adequate therapy minimizes the hazard of drug toxicity. The low value of ERPF occasionally observed immediately after transplantation is believed to represent ischemic damage.

Although kidney transplants between a mongrel population might be expected to exhibit wide variation in rejection times, two consistent pathophysiological patterns were observed in both the modified and unmodified hosts. The rapid rejectors uniformly exhibited a simultaneous and early decline in ERPF and GFR. They were also the most difficult to obtain prolonged graft survival. It is suggested that these results are best explained by the degree of histoincompatibility between donor kidney and host.

In this study, ERPF was estimated by the single injection of 113H-hippuran and GFR by the single injection of Tc99m(V)-citrate complex of creatinine clearance as reported earlier (Blafox and Merrill, 1966; Kountz et al., 1967). The use of Tc99m(V)-citrate complex for estimation of GFR by the single injection technique avoids urine collections and allows estimation of GFR in uremic subjects early after transplantation when the serum creatinine is declining.

The most impressive aspect of this study was the superior early function and survival of grafts that received local treatment, and in general less immunosuppressive therapy. In both animals and man the results were better and toxicity less. There is little information on the mechanism of drug action in prolonging the survival of renal allografts, although azathioprine has been shown to have a cytotoxic effect on specifically sensitized lymphoid cells or homologous target cells in culture as demonstrated earlier (Wilson, 1965). Actinomycin-C and azathioprine have been shown (Retik et al., 1967) to reverse incipient rejection when administered directly into renal allotransplants.

The pathophysiological pattern of the first rejection appeared to determine the fate of the grafts. Local therapy always prolonged the first rejection when administered immediately after grafting but in many cases was unable to completely abolish it. The long-term success of a renal transplant depends on a close titration with immunosuppressive drugs—too much results in death from toxicity and too little in death from uncontrolled rejection. The ease
with which stability of ERPF and GFR can be achieved with small and safe doses of immunosuppressive therapy might well depend on the degree of histoincompatibility between donor and host. A correlation of these pathophysiological groups with leukocyte antigen matching is in progress.

Summary

Two pathophysiological patterns in dogs and three in man with renal allotransplants on immunosuppressive therapy have been observed at the time of the first rejection. In dogs: (1) the rapid rejectors with an early and simultaneous decline in ERPF and GFR; and (2) slower rejectors with a sequential decline in ERPF and GFR. In man: (1) rapid rejectors with an early and simultaneous decline in ERPF and GFR; (2) slower rejectors with a sequential decline in ERPF and GFR; and (3) those with long stability of ERPF and GFR. Serial measurements of ERPF, when performed daily, starting immediately after transplantation when there is little ischemic damage from grafting, proved to be a valuable diagnostic and therapeutic tool. Elevation of the serum creatinine followed declines in ERPF and GFR. Initial local intra-renal treatment appears to eliminate in man the rapid rejectors with an early and simultaneous decline in ERPF and GFR and prolongs the first rejection in dogs. Superior initial graft function and prolonged survival were noted in the locally treated transplants with less immunosuppressive therapy.

REFERENCES