OBSERVATIONS ON THE EARLY DIAGNOSIS AND CHEMOTHERAPEUTIC TREATMENT OF THREATENED RENAL REJECTION IN DOGS

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Technically the transplantation of tissues and organs presents little difficulty.[1,2] Skin grafting is now an everyday procedure. The homotransplantation of kidneys has been done successfully in man since 1958 and even organs such as the lungs, liver and heart have been transplanted in experimental animals. Both clinically and experimentally the outcome of such homotransplants has been universally unsuccessful after a variable period of reasonable function. The rejection, or 'biological incompatibility', of homotransplanted kidneys is in marked contrast to the continued function of kidneys transferred from one site to another in the same animal (autotransplants).

In 1945 Medawar and others[3-10] developed and propounded the current idea that homograft rejection is the result of an immune reaction in the host against the antigens of the graft, and that a second graft from the same donor is rejected at a faster rate than the first—the 'second set reaction'. He also demonstrated that variation in the rate of graft breakdown is dependent on the degree of antigenic difference between host and donor and is not primarily related to the graft location, the grafting technique or number of sites.

In a homotransplant reaction, antigenic material—in all probability a small basic protein complex[11]—is released from the graft site. The immunologically competent cells of the host, present in any lymphoid tissue or organ, proliferate in response to such a stimulus and the resulting cell population (predominantly round cells) exhibit immunological specificity for donor antigen. Such cells return to the graft site, interact with donor antigen, and cause all the clinical and pathological signs of the rejection phenomenon.

The aims of this series of experiments were:

1. To study the rejection phenomenon;
2. To diagnose rejection at the earliest possible stage; and
3. To prevent or reverse rejection by means of cytotoxic agents.

MATERIAL AND METHODS

Operative Technique[12]

Adult mongrel dogs weighing between 35 and 45 lb. are anaesthetized with pentobarbitone sodium and intubated, and ventilation is maintained manually with a mixture of nitrous oxide and oxygen. The kidney of the donor animal is approached through a right subcostal muscle-splitting incision. The right kidney is preferred, there being a much lower incidence of anomalous renal arteries on that side. Nephrectomy is performed after mobilization of as long a length as possible of both renal artery and vein, with division of the ureter approximately 10 cm. from the renal pelvis, without ligation of the ureteric artery proximal to its division.

The capsule of the kidney is incised and stripped along its length. A blunted size 15 needle is introduced into the renal artery and the kidney perfused under low pressure until the return from the renal vein is clear. The perfusate consists of 250 ml. of normal saline, 25 ml. of 1% Leostesin and 1·5 ml. of heparin (30 mg.).

When the donor animal has been bilaterally nephrectomized, an incision of 10 cm. is made to the left of the midline in the mid-cervical region. The external jugular vein and common carotid artery are both dissected free and ligated distally. The renal artery and vein are anastomosed end-to-end to the common carotid artery and external jugular vein respectively (Fig. 3) and a small cavity for the kidney is constructed by blunt dissection deep to the panniculus carnosus, lateral to the skin incision. Great care is taken to avoid kinking of the renal vein. A cutaneous ureterostomy is then created in the midline of the neck.

With this technique, renal ischaemia averaged 20 minutes and in this study never exceeded 35 minutes.

There were 3 groups of dogs in the series:

Group 1. Renal autotransplants (6 dogs)—right kidney transferred to the neck of the same animal with a simultaneous nephrectomy.

Group 2. Renal homotransplants without cytotoxics (14 dogs)—donor kidney transplanted into a bilaterally nephrectomized recipient.

Group 3. Renal homotransplants with immuno-suppression (10 dogs)—using the same technique as in group 2, with modification or prevention of repudiation by the use of immuno-suppressive drugs.

Drug Therapy[13-21]

1. *Imuran* (*6-mercaptopurine derivatives; aminothioprine*). Currently the most widely used of the immuno-suppressive agents, this is a purine analogue antimetabolite. *Dosage*: 4 days pre-operatively 2 - 4 mg./kg.; day of operation and 1st day postoperatively 8 mg./kg.; time of rejection and subsequent day 10 mg./kg.

2. *Actinomycin C.* Both antibiotic and cytotoxic, this is administered intravenously in a single dose of 200 μg. at the time of rejection. If necessary the dosage may be repeated within 4 - 7 days.

3. *Prednisone* is instituted only on the diagnosis of rejection phenomena, with a standard procedure for gradated withdrawal. Its mode of action is both anti-inflammatory and lympholytic.

4. *Prophylactic antibiotic* cover with penicillin and streptomycin is provided for 4 days from the day of operation and for the same period on detection of graft rejection.

Intravenous fluids are infused postoperatively for 8 days to ensure: (1) adequate urinary output in an attempt to prevent urinary tract infection, and (2) proper hydration; 500 - 1,000 ml. of either half-normal saline or a balanced-electrolyte solution are used.

Special Investigations

The following investigations are performed daily, commencing 3 days before surgery and continuing until the animal dies or is sacrificed:

(a) *Urine*: volume, specific gravity, protein, catalase, stained preparation of urinary sediment.
(b) *Blood*: haemoglobin, white cell count, differential count, ESR.
(c) *Both blood and urine*: sodium, potassium, chloride, urea, creatinine.
(d) *Temperature, pulse and blood pressure.*

RESULTS

Before discussing the value of individual investigations in the proper understanding of either the rejection phenomenon or its early diagnosis, it should be stressed that, other than a transient elevation of blood urea for approximately 7 days postoperatively, group 1 dogs (autotransplants) behaved as normal animals. This was conclusive proof of the adequacy of the surgical procedure.[22]

The only results discussed below are those of investigations which proved to be helpful.

Urinary Volume

Immediate renal function is of great significance in evaluating technical success.[23] Such factors as prolonged kidney ischaemia, or narrowing of either the arterial or venous anastomosis, adversely affect the onset of renal function, urinary-output volume and other parameters of renal function. Immediate urinary output was the rule in all these experiments. Possibly as a result of the daily intravenous infusions, urinary volume was not found useful in the diagnosis of rejection. Certainly in group 2 (homotransplants without cytotoxic treatment), some days after rejection had been diagnosed urinary volume steadily diminished, culminating in death from complete renal shutdown. In the dogs treated with immuno-suppressive drugs (group 3) no distinctive pattern of either increase or decrease in urinary output was evident at any stage.

Urinary Protein (Figs. 1, 2)

In all 3 groups transient proteinuria was present for 24 - 48 hours postoperatively. Its increase (or re-appearance) heralded the onset of rejection and was considered important in the diagnosis of this phenomenon.[15] For obvious reasons, in both infected and blood-stained urine, protein estimation was of no value.

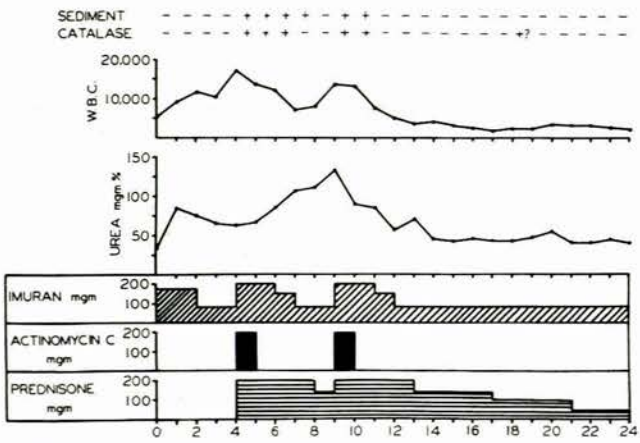


Fig. 1. Renal homograft using cytotoxics. Note dramatic response of renal function to immuno-suppressive therapy (Dog 137, weight 41 lb.).

Stained Urinary Sediment (Figs. 1, 2)

A spun deposit of urinary sediment was stained and was diagnostic of kidney rejection.[24] Lymphocytes appeared in clumps, singly and as casts, and persisted until either the animal died or until rejection had been adequately reversed (Figs. 4, 5). Coincidental with these lymphocytes, monocytes, red blood cells, tubular epithelial cells and tubular casts were seen.

Comparing groups 2 and 3, there was a quantitative difference in the numbers of these cells. The sudden appearance of 'flushing' of the urine was most striking and was never seen in autotransplanted animals.

Urinary Catalase[25,26] (Figs. 1 - 3)

Renal tissue is rich in catalase, a reducing enzyme, although in normal circumstances it is never present in the urine. This was a qualitative test, always confirmatory when rejection was thought to be occurring but open to false positive results when haematuria or infection was present. A quantitative procedure is now being developed.

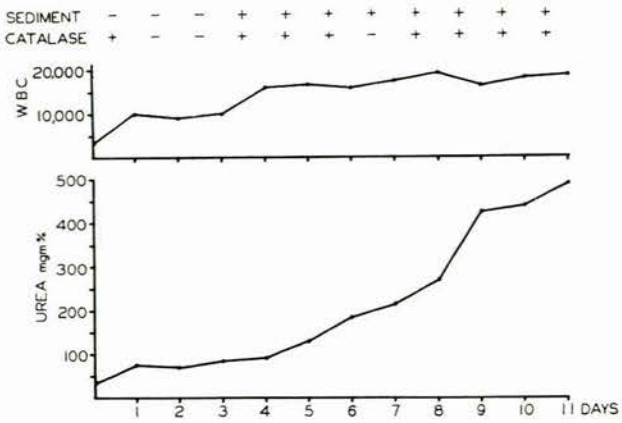


Fig. 2. Renal homograft without cytotoxics. Note progressive deterioration of renal function, culminating in death at 11 days (Dog 85, weight 45 lb.).

White Cell Count and Differential Count (Figs. 1, 2)

There were 2 reasons for accurate daily estimation of these parameters:

1. To assess the cytotoxic effects of immuno-suppressive drugs on bone marrow, and
2. To confirm rejection.

In all 3 groups the white cell count was raised for 2 - 3 days postoperatively with a predominant polymorphonuclear response—a normal reaction to surgical trauma.

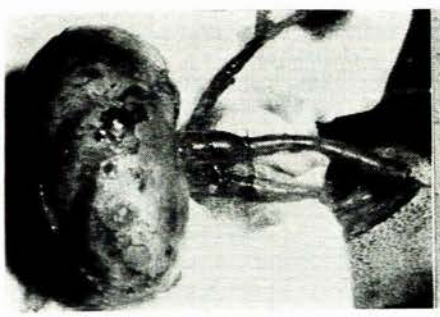
Leucopenia, to a greater or lesser degree, developed in all dogs treated with cytotoxics;[27] this was related to the massive dosage used at the time of operation and again at the time of rejection, often twice in the latter case. In none of the group 3 animals was the leucopenia reversed and this provided a satisfactory but highly dangerous index of increased susceptibility to infection. Conversely, however, it was of no value in gauging the suppression of immune responses.[19,21]

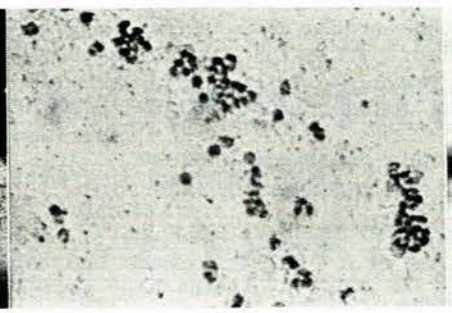
At the time of rejection there was a dramatic increase in total white cell count.[28] A quantitative difference between groups 2 and 3 existed, but in both the increase was relatively similar. In untreated dogs (group 2) the white cell count remained markedly raised with a pronounced eosinophilia in addition to accompanying polymorph response. This has been noted by other workers and its absence is ascribed to the use of steroids.[29] With reversal of rejection in treated dogs (group 3) the total white cell count was reduced immediately and there was a relative decrease in polymorphs and an increase in lymphocytes.[30]

At later stages in treated dogs (group 3), there was a noticeable diminution in the number of platelets with anisocytosis and poikilocytosis of red cells.[19]

Serum and Urinary Electrolytes and Creatinine

These afforded little or no help in the early diagnosis of rejection.[24] In many group 3 dogs slight sodium retention occurred over the rejection period.[29,30] As complete renal shutdown became manifest in untreated animals,





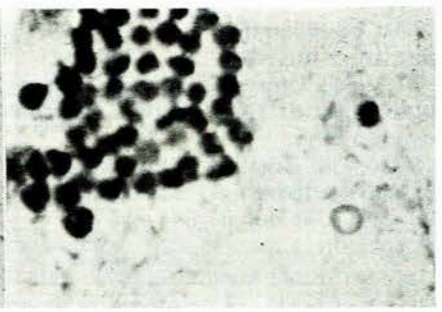


Fig. 3 *Fig. 4* *Fig. 5*

Fig. 3. Completed anastomosis of renal artery and vein to common carotid artery and external jugular vein respectively. Note capsulotomy and immediate flow of urine. The urine is heavily blood-tinged from an unligated ureteric artery.
Fig. 4. Photomicrograph under low-power magnification of clumps of lymphocytes in stained urinary sediment. Note occasional epithelial and red blood cells.
Fig. 5. Photomicrograph under high-power magnification of a single clump of lymphocytes.

gross abnormalities (consistent with renal failure) ensued.

Technical difficulty in proper urinary collection made creatinine clearance estimation an infrequent investigation. This would seem to be a reasonably sensitive index of rejection, particularly in human transplantation.[19,23,29]

Blood Urea (Figs. 1, 2)

(*a*) A transient rise occurred for 1 - 2 days postoperatively.

(*b*) A variable but marked rise resulted, usually 24 - 48 hours after the onset of rejection.[19,23,27,29,31]

1. Untreated animals continued to show further increases, to astronomical levels, before death.
2. Treated dogs had an inconsistent response. In some elevation persisted until a second episode of rejection was treated, at which time there was a gradual return to normal. Whether this prolonged elevation was part of persistent rejection, or of inadequate treatment, is difficult to assess.[29] In others, a return of blood urea to normal occurred after treatment, with a similar train of events at a second rejection.
3. Without exception blood urea was eventually within normal limits in all treated animals.[29]

X-ray Examination (Figs. 6, 7)

Intravenous pyelography and renal artery arteriography were carried out in group 3 animals when rejection had been successfully reversed and blood urea was within normal limits. A comparison with autotransplanted group 1 dogs failed to show any noticeable difference.

Graft Size

Rejection was associated with a remarkable increase in size of the homografted kidney.[19] After adequate immunosuppression, the graft size gradually returned to normal. In untreated animals, further enlargement occurred until the animal's death.

General

(*a*) The immuno-suppressive drugs did not produce any apparent symptoms in the gastro-intestinal tract.

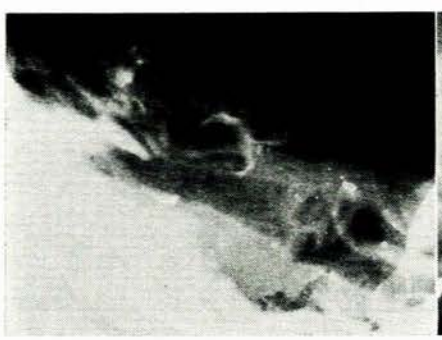
(*b*) Uraemia was responsible for severe anorexia, nausea and vomiting.

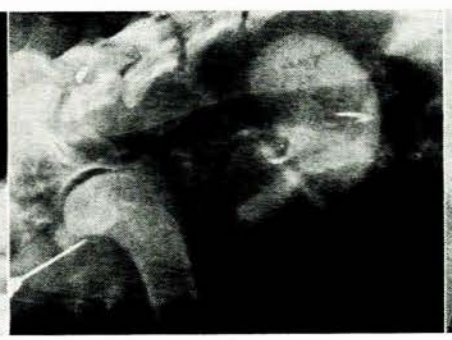
(*c*) Rejection was associated with hyperpyrexia, tachycardia and marked listlessness. Hyperpyrexia was considered important confirmatory evidence,[19,28,29] and was an invariable occurrence—not, however, at a significantly early stage.

(*d*) Blood pressure readings were, if anything, misleading.

Over-all Survival[19,21,32,33]

Group 1. Autotransplants (total: 6 dogs). One died on





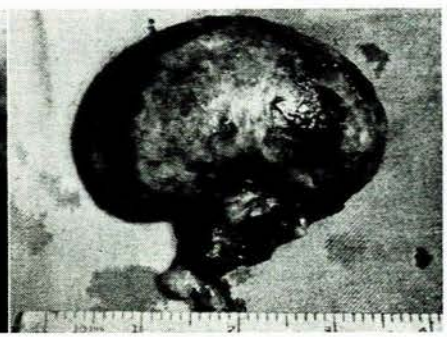


Fig. 6 *Fig. 7* *Fig. 8(a)*

Fig. 6. IVP of a treated homotransplanted kidney at 25 days. At this stage other parameters of renal function were within normal limits. Essentially normal with no sign of ureteric obstruction.
Fig. 7. Nephrogram of a treated homotransplanted kidney at 26 days. Renal artery was of normal size and vascularization of the kidney was both prompt and uniform. Note renal outline, normal in both size and shape.
Fig. 8. Macroscopical views of a rejected homograft kidney. (*a*) Gross swelling with marked evidence of subcapsular haemorrhage.

the 1st postoperative day as a result of haemorrhage and there are 5 survivors.

Group 2. Untreated homotransplants (total: 14 dogs). Survival varied from 6 to 11 days, the average period being 8·5 days.

Group 3. Treated homotransplants (total: 10 dogs). Survival varied from 14 to 26 days, the average being 20·1 days. One dog is alive and well at 42 days.

Causes of Death

(a) *Untreated homotransplants*—all the animals died as a result of renal failure caused by rejection.

(b) *Treated homotransplants*[34]—these animals died of septicaemia (4), pneumonia (3), peritonitis (1) and volvulus (1).

None of these animals succumbed as a direct result of renal rejection. In all except one case infection supervened and, with lowered resistance, death ensued.

PATHOLOGICAL FINDINGS

Group 2 — Untreated Dogs (*Figs. 8a, 8b*)

Macroscopic appearance. Grossly, all transplants showed marked enlargement and engorgement.[35] Several different patterns were noted on the cut surface, varying from marked haemorrhage at the cortico-medullary junction, to oedema and focal haemorrhage. The diversity of the gross picture was thought to be due to the following variations:

1. The rapidity of rejection—an index of genetic dissimilarity.
2. The degree to which one microscopic element develops relative to another.
3. The stage of destruction at postmortem.
4. The reaction of the transplant against the host's antigen.

Microscopic appearance.[36-39] The homotransplanted kidney in the dog shows striking changes which are always more pronounced in the cortex:

1. Round cell proliferation and infiltration (Fig. 9).
2. Swelling of the endothelium of the small vessels of the cortex (Fig. 10).
3. Differentiation of the endothelial cells into plasma cells.
4. Interstitial oedema.

These changes progressed to:

5. Avascularity of the glomeruli.
6. Focal areas of interstitial haemorrhage.
7. Dilatation of the tubules and cast formation.
8. Degeneration of the tubular epithelium.

Group 3 — Treated Dogs[39]

At autopsy, the graft was essentially of normal dimensions and on section no obvious abnormality could be detected (Figs. 11a, 11b). The cortico-medullary junction was intact and there was no visible haemorrhage, infarction or necrosis.

Microscopic. In complete contradistinction to group 2 animals, the picture was in most cases indistinguishable from normal (Fig. 12).[40] In some an insignificant cellular infiltrate persisted, but in general the glomeruli, tubules, interstitium and arterioles were within the range of normality.[41,42] There was no evidence of the obliterative vascular change that occurs late in human transplants.

Another invariable finding was a small contracted spleen which, histologically, was replaced largely by fibrous tissue.[35] Although there was no control group of normal animals on cytotoxics, this was presumably the immuno-suppressive effect on a highly immunologically competent organ.

Note on Cellular Infiltrate

In 1953 Dempster and Simonsen[43,44] stated that pyroninophilic cells and plasma cells were derived from the transplanted kidney as graft against host reaction. Porter and Calne[45] labelled the prospective host dogs with tritiated thymidine before kidney transplantation and observed heavily labelled host cells in the kidney. They concluded that the cells infiltrating homografted kidneys were derived from circulating lymphocytes from the host.

Dempster and Williams,[46] also using tritiated thymidine, in 1963 postulated that there were 2 populations of cells differentiating within the kidney homograft, 1 of host origin and 1 of renal origin. They concluded that the relative magnitude of the 2 populations was not known. In addition, Yoshii's investigations on the origin of these monocytic cells, using a sex chromatin label,[47] was compatible with the derivation of the infiltrate from both host and recipient, the greater number of cells being of host

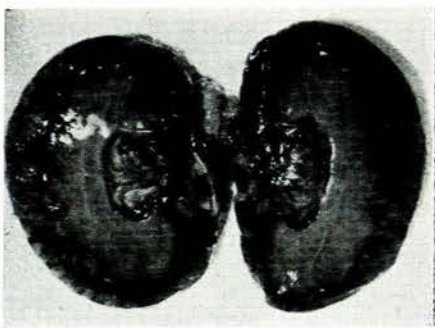


Fig. 8(b)

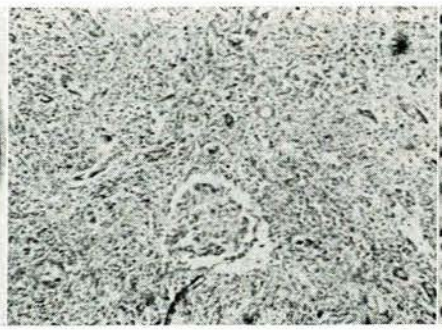


Fig. 9

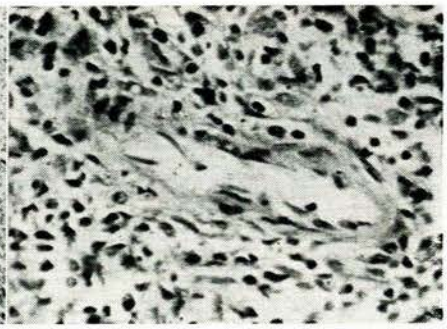


Fig. 10

Fig. 8(b). Cut surface shows enlargement, evidence of cortico-medullary haemorrhage together with oedema and stippling of both cortex and medulla with predominantly cortical areas of focal haemorrhage. There is nothing to suggest hydronephrosis.

Fig. 9. Photomicrograph of the cortex of a rejected kidney. Note the extensive round cell infiltration, interstitial oedema, tubular degeneration and ischaemic glomerulus.

Fig. 10. Photomicrograph of renal arteriole. There is marked round cell infiltration and interstitial oedema with fibrinoid necrosis, swelling of and disruption of the vessel wall.

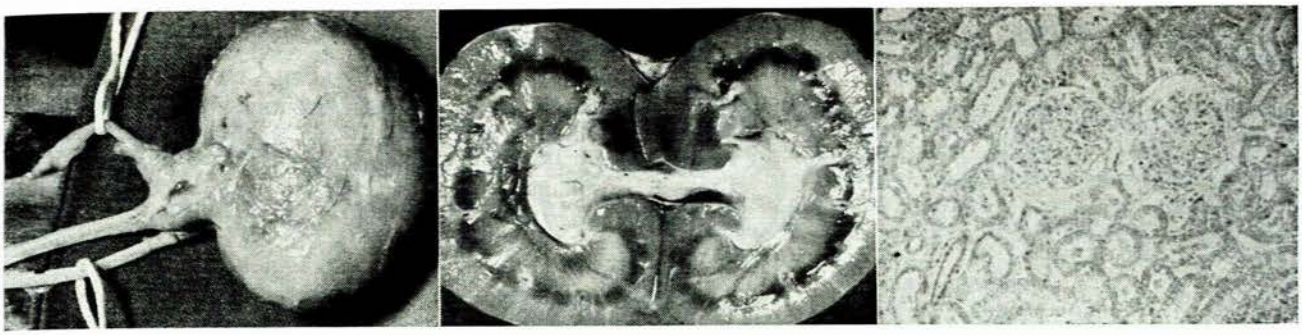


Fig. 11(a) *Fig. 11(b)* *Fig. 12*

Fig. 11. Macroscopical views of a treated homotransplanted kidney at 51 days. (*a*) Normal size; no evidence of subcapsular haemorrhage or infarction. (*b*) Cut surface in contradistinction to Fig. 8(*b*) is essentially normal.
Fig. 12. Photomicrograph of the renal cortex of a treated homotransplanted kidney at 21 days. Note virtual absence of cellular infiltrate and interstitial oedema. Tubular and glomeruli appear normal (compare with Fig. 9).

origin and the lesser from donor reticulum and vascular endothelial cells. The proliferating cells of the homografted kidney represent a reaction of the graft against the host.

DISCUSSION

The diagnostic importance of early graft rejection lies in the assumption that it coincides with the onset of the first pathological signs of rejection, foregoing any irreversible effect.[18] From serial biopsies in untreated homotransplants, there is no question as to the progressive nature of all that rejection entails.[35] Equally certain is the fact that this progression cannot only be arrested, but is also reversible by the use of immuno-suppressive drugs.[37] It is the contention that the sooner reversal measures are instituted, the better is the ultimate prognosis for that kidney.

The appearance of lymphocytes in a stained preparation of urinary sediment was the most valuable single investigation in early diagnosis.[24] Interpretation of the smears did not provide great difficulty and invariably was conclusive. In Table I are the diagnostic criteria of renal homograft rejection.

TABLE I. DIAGNOSTIC CRITERIA OF RENAL HOMOGRAFT REJECTION

1. Abnormal urinary sediment stain.
2. Decreased total urine output.
3. Unexplained temperature elevation.
4. Increase in size of homograft with pronounced tenderness.
5. Increase in proteinuria.
6. Rise in blood urea.
7. Decrease in renal clearance values.
8. Positive urinary catalase.
9. Increased total white count with marked polymorph preponderance.

The differential diagnosis between incipient immunological rejection and infection is difficult.[48] Lymphocyturia proved not only invaluable in diagnosing rejection, but also in its differentiation from infection when the great majority of other signs proved to be inconclusive.

We feel that the diagnosis of graft repudiation can be made with confidence (Table I). With satisfactory urinary collection over long periods, clearance values became operative and, in our limited experience, should be of benefit.[25] Reports of more complex parameters of renal function, including radioactive renograms[30] and other enzyme assays,[49,50] indicate that these too are of importance. It must be emphasized that all these clinical parameters are in themselves valuable guides, but the final decision of active rejection is a summation of all these factors and their rates of change in response to drug management.[51] Although urinary sediment was considered the single most important evaluation, it was never a solitary positive finding.

Abnormal urinary sediment constituents were apparent in untreated animals from the 2nd/3rd postoperative day. By the 4th/5th day vast numbers were observed. With suppression of rejection, lymphocyturia was first seen on the 4th/5th day, there being a striking quantitative discrepancy when compared with untreated animals at the same stage. In the majority of treated dogs a second episode of threatened rejection was apparent between the 10th and 15th days; this has already been discussed.

Assuming the early appraisal of rejection to be valid, immuno-suppressive drugs do not cause a significant delay in the immunity response in dogs.[31] In human transplantation, especially in the presence of close consanguinity, delay of graft rejection (if not prevention) is not uncommon.[29,52]

Rejection Not Uniform

For ill-understood reasons, the severity of rejection is not a uniform manifestation of dogs. This is exemplified by:

(*a*) The rapid progression to complete renal shutdown in some untreated animals, whereas in others renal function was impaired at a far later stage.

(*b*) The ease with which immuno-suppressive drugs reverted graft rejection in the minority of treated dogs, compared with continued evidence of repudiation in others, necessitating higher dosages before eventual reversal was attained.

Part of the explanation lies in variation in the degree of antigenic dissimilarity and part in the inconsistency of action of the cytotoxic drugs.[17,53,54]

At the present time there appear to be 4 empirical approaches to the conditioning of recipients for homotransplantation:

1. Total body irradiation at 'sublethal' doses. The published results with this modality are not favourable.[55-58]

2. Combinations of total body irradiation and chemical suppression.[29,59]

3. Focal irradiation to, or surgical removal of, specific lymphoid tissues.[29,60-62]

4. Chemical inhibition by immuno-suppressive drug therapy.[14-21,29]

Drug Therapy

There are many major advantages of drug therapy for the suppression of immunity:[19,51,63,64]

1. Continuous inhibition of the immune response.
2. The therapy can be instituted either immediately before transplantation or after the transplant has been completed.
3. The extraordinary environmental precautions necessitated after total body irradiation can at least in humans be supplanted by minimal precautionary measures.[56,57,65,66]
4. Immuno-suppressive drug therapy does not completely inhibit the host immune reaction.

The prolonged survival of canine renal homografts produced by immuno-suppressive drugs may be explained by:[18,21,31,32,67]

(*a*) Complete suppression of the host immune response, making the animals immunological cripples;

(*b*) Partial but specific abrogation of the host immune response;

(*c*) Antigenic adaptation of the graft to the host;

(*d*) Immuno-paralysis induced by antigen overloading from the surviving kidney; and

(*e*) Some other as yet unexplained mechanism.

Various reports suggest that drug-induced immunological tolerance is a delicately balanced state specific for the antigen introduced at the time of transplant.[32,68] Antigenic adaptation of the graft to the host does not occur because the kidney can be returned to its immunologically competent original host without rejection.[20] Drug-induced tolerance is not permanently drug-dependent,[18,67] although it may be so for a prolonged period, nor is it dependent on alteration in the metabolism, absorption or excretion of the drug.

Theory

A theoretical explanation of graft tolerance induced by drugs may be as follows:[40,68-70] The inhibition of the inductive phase of antibody formation by the antimetabolite. Although this inhibition is not complete, the renal homograft is able to withstand the markedly reduced hypersensitivity response (antibody formation) because of its organ size (antigenic mass) without functional impairment. The size of the transplanted graft is critical as far as prolonged survival under these circumstances is concerned. Thus the host's immune response is ineffectual in destroying the transplant. In the presence of the antimetabolite, sensitized lymphoid cells are no longer induced; those present may be killed by the antimetabolite as they proliferate in regional nodes and in the renal homograft, or just live out their natural life-span. Thus the immature lymphoid cells mature in an environment in which they recognize the transplant as self, and no future sensitization takes place. Once the sensitized cells have disappeared, there is no further need for the antimetabolites. The longer the homograft persists, up to an undefined critical point, therefore, the greater the opportunity for adult-acquired tolerance to be achieved in the presence of the antimetabolite.

In this series of experiments we have attempted to translate a system of human rejection therapy into canine transplants. From the foregoing figures this can hardly be said to have been successful. Throughout, drug dosage was empirical and without exception toxic bone marrow depression and increased susceptibility to infection was provoked.[37,34,71] Only one animal survived 30 days despite profound leucopenia. At the time of writing a slight improvement in total white cell count is shown, but it must be most unlikely that this can ever revert to normal. With the development of any intercurrent infection, the chance of survival is minimal. Not one animal in this series died directly from the effects of homograft rejection. In all infection supervened and could not be controlled.

A recent shift in homograft philosophy has resulted in the use of minimal drug dosage to allow incipient rejection, which might then be treated.[72] The lethality of drug over-dosage, certainly operative in these experiments, is clearly demonstrated.[29,34,73] Imuran is singly the most important drug with Actinomycin C and cortisone reserved for reversal of threatened rejection.[19]

In recent times less emphasis is being attached to complete isolation techniques in the handling of drug-treated human recipients.[72] Without argument, this is an encouraging aspect of drug therapy. What must not be forgotten, however, is that the manipulation of immuno-suppressive drugs is, in these cases, the responsibility of highly experienced transplant experts.

Successful human kidney transplantation is universally the product of intense and diligent experimentation, of sound understanding of the immunological principles involved, of the realization of the limitations of present-day therapy, and of the inherent dangers of such treatment. Unless these criteria are fulfilled and unless hospital facilities are unimpeachable, no surgeon has the right to subject his patients to renal homografts. The corollary to this suggests that the future transplant surgeons should avail themselves of research facilities and should fully realize the magnitude of their tasks before applying their skills to clinical practice.

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