THE DEVELOPMENT OF IMMUNOSUPPRESSION IN THE RENAL MODEL

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I would like to talk about the development of immunosuppression as this occurred with renal transplantation and then was applied to the transplantation of other organs. This was an entirely natural sequence because with the other organs (liver, lung, heart, heart-lung, and pancreas grafts) the technical requirements and technical complications were so high that the evaluation of new immunosuppressive drugs was not really feasible.

With the simple kidney transplantation model, it was possible to define the patterns of rejection without the artifacts caused by surgical complications and to assess how immunosuppression changed these patterns.

CELL MEDIATED VERSUS HUMORAL REJECTION

The collateral issues of typing which you heard about this morning also were analyzable only in the simple renal model. It became obvious in the early 1960's that cell mediated rejection was not the only kind of immunologic problem which we had. This morning, James Cerilli talked about the fact that in hyperacute rejection the signal event is devascularization of the kidney cortex despite the main renal vessels being open. It was recognized that hyperacute rejection was precipitated by antibodies such as the isoagglutinins that attach to renal cells if transplantation is performed across red blood cell group barriers (1) or more importantly if the recipient has antigraft cytotoxic antibodies (2). The avoidance of hyperacute rejection is not dependent upon immunosuppression but rather on the avoidance of antibodies by tissue typing.

MODIFIED CELL MEDIATED REJECTION

In 1962 and 1963 it was recognized that azathioprine and prednisone could be used together to modify cell mediated renal rejection. In Figure 1 are shown the events following transplantation from a brother who probably was well matched at the A, B, and DR loci although we did not know this at the time. The creatinine clearance which was near zero before went to super normal levels after operation. The recipient had a massive diuresis which was typical in those days because of the generally poor condition of the recipients which in turn was explained by the fact that chronic hemodialysis was not generally available. The patient had a magnificent recovery and felt better for about 2 weeks than he had for several years.

The sense of well being was temporary. Secondary deterioration of graft function followed with a rise in BUN, and a decline in creatinine clearance. A finding that is not much seen any more because of the extensive use of steroid therapy today was fever (Figure 1). Also, the patient gained weight and developed proteinurea. In our earliest kidney recipients, azathioprine was used alone at first (Figure 1) and steroids were reserved to treat proven or presumed rejection (3).

With the institution of prednisone therapy (Figure 1), renal function improved and the other adverse findings including fever were ameliorated. As these patients were successfully treated, it was realized that rejection was a reversible process (1, 3). An additional interesting observation in some of these early

patients was that it became possible to greatly reduce or in a few instances to even stop the prednisone therapy within a surprisingly short time. This implied the induction of an altered host-graft relationship which we rashly called "tolerance" (3). The kidney whose function is depicted in Figure 1 is still functioning more than 20 years later.

ALTERNATIVE IMMUNOSUPPRESSIVE REGIMENS

Experiences in 1962 and 1963 such as those shown in Figure 1 constituted the beginning of the so-called double drug therapy with azathioprine and prednisone that has become the standard throughout the world. Before this time, 6 mercaptopurine and azathioprine had been used as single agents, but the success rate was miniscule (4).

Subsequently, a number of deviations from the original double drug programs have been described (Table 1), as summarized elsewhere (5). Perhaps the most important was the use of antilymphocyte globulin (ALG) as adjunctive therapy during the first few postoperative days or weeks (6). The addition of ALG to base therapy with azathioprine and prednisone has been called "triple drug therapy". It was of considerable interest to note a few years later that cyclophosphamide, the widely used anticancer agent, could be substituted freely for azathioprine (7) (Table 1). Cyclophosphamide had been (and is still) thought to be a fairly specific drug against B lymphocytes for which reason some people thought it surprising that the drug was as effective as the azathioprine to which anti-T-lymphocyte activity had been attributed.

Prior to 1962, the literature about renal transplantation was uniformly pessimistic in all except twin cases. For this reason, it was remarkable how well our first wave of patients did under treatment with azathioprine and prednisone. After consanguineous transplantation (excluding twin cases) in 1962 and 1963, the one year graft and patient survival was almost 70% (1). More than half of the kidney grafts were still functioning at 10 years (8) and now with 20 years of followup the number is still almost half.

It was interesting that in our subsequent experience (1964-1966) using double drug immunosuppression for consanguineous transplantations was not quite as good in spite of the fact that an effort was made to prospectively tissue match all donors and recipients (8). These disappointing results were prophetic of those in later and much larger trials which also showed that tissue matching (at least at the A and B loci) was a poor instrument of donor and recipient selection except for sibling combinations.

The use of the triple drug combinations provided better results after related transplantation and it became common year after year to have graft survival after related transplantation at or above 80% (8).

THE NON-RELATED DONOR

The defect in renal transplantation and one which of course was transfered to all extrarenal organs was that the results were so poor after cadaveric transplantation or transplantation from

living non-related donors. In our 1962-63 series, two thirds of the recipients of non-related kidneys died during the first postoperative year of graft rejection or of complications of the immunosuppression used to control the rejection (1). Most of these donors were living related volunteers, and thus the quality of the grafts was generally better than could be obtained under the condition of cadaveric donation which pertained in those early years. At that time, chronic dialysis was not generally available, and because of this, patient and renal graft survival were very nearly synonymous.

The one year survival after transplantation from nonrelated volunteers or cadaveric donors in our Series 2 (1964-1966) rose to 50%. In subsequent series from 1966 to 1972 in which the triple drug programs were used, including ALG, the one year patient survival rose to the more satisfactory levels of 80% or better (8). However, this increased survival was explained in part by the more and more common practice of returning patients to dialysis in the event of an unusually hard rejection; many of these patients underwent retransplantation (8).

During the decade beginning in 1970 it became a common practice to look at graft (not patient) survival in assessing the effectiveness of immunosuppression. In this same decade, there was a drying up of reports of cadaveric renal transplantation from individual centers. I suspect that the reason was that many surgeons who were using double drug therapy were having such poor graft survival that they labored under the impression that other

people must be doing better. This perception of things was undoubtedly aided by a tendency from a few centers to issue what have been termed "See what a big boy am I" reports which at times were based upon incomplete data or upon data pools that were diluted by unspecified numbers of related transplantations in addition to the cadaveric cases.

The true state of affairs was revealed by reports from Dr. Paul Terasaki's center at the University of California, Los Angeles. Terasaki provided a mechanism for more than 100 centers to report their results under a cloak of anonymity. It was found that the one year cadaveric graft survival under conventional (for the most part double drug) therapy was 50% or less (9). As recently as 1981, another multicenter report from the Southeastern Organ Procurement Foundation has shown the same thing (10).

Finally, reports from centers known for the quality of patient care such as the Peter Bent Brigham Hospital, showed one year cadaveric kidney survival of considerably less than 50% in recipients who were surviving for one year at better than a 90% rate (11). Individual centers which had higher cadaveric graft survival almost invariably paid a price of an increased one year patients mortality (12). Thus differences in graft survival from center to center reflected in part differing philosophies about what kind of patient mortality to accept, and the extent to which immunosuppression was pushed to the limit.

THE WATERSHED YEAR OF 1978

The need for fundamental changes in immunosuppression or some other aspect of the strategy of cadaveric transplantation was widely acknowledged by the time the International Transplantation Society met in Rome in early September, 1978. The possible value of matching at the DR locus was at center stage for the first time, and in addition Terasaki's concept of recipient preparation with multiple blood transfusions (1, 3) had been increasingly accepted. However, both of the foregoing approaches would have tended to restrict the numbers of patients treated with transplantation.

In particular, it was obvious that the practice of preoperative transfusions improved the statistics after cadaveric transplantation but at the cost of rendering many patients nontransplantable who developed widely reacting cytotoxic antibodies. What was happening was that part of the "transfusion effect" was the weeding out of strong immunologic responders. The transfusion approach had the capability of making the transplant surgeons' statistics look better, but the aims of society partially were being subverted by consigning a significant number of patients to permanent dialysis.

In the field of immunosuppression, three major topics dominated the 1978 meetings. One was the use of total lymphoid irradiation for preoperative recipient preparation. The techniques had been worked out at Stanford University by Strober et al (14) and the first clinical trials had been begun at the

University of Minnesota (15). A second technique was also based on lymphoid depletion prior to transplantation and was a re-examination of thoracic duct drainage (TDD) (16) which was first used clinically by Franksson of Stockholm more than 15 years earlier (17).

The earlier trials of thoracic duct drainage had not been successful, partly because the pace of the immunologic changes caused by TDD in humans was not understood. In his original studies in rats, James Gowans of Oxford had shown profound immunodepression within 5 days after beginning TDD and it was assumed that the same applied in humans. It was not until the late 1970's that it became clear that 20 to 30 days of effective thoracic duct drainage was necessary in man before an advantage was created for a new transplant (16).

The necessity for such a prolonged preparation for cadaveric transplantation implied a high cost and excessive inconvenience. In spite of these disadvantages, thoracic duct drainage undoubtedly would have undergone a clinical renaissance were it not for the fact that the possibility of better drug therapy also came to the fore at the same time. The incidence of rejection with appropriate TDD pretreatment was reduced to less than 5% in the first three months after primary cadaveric transplantation (16).

The most important subject at the 1978 Rome meeting was the potential value of the new immunosuppressive drug cyclosporine which had been discovered by scientists at the Sandoz

Corporation, Basel, Switzerland. The immunosuppressive qualities of cyclosporine had been described by Borel et al (18). The drug was capable of inhibiting a number of experimental auto-immune diseases and was spectacularly effective in preventing skin graft rejection in rodents. The drug was described as having weak myelotoxicity, and subsequent observations have suggested that there may be no bone marrow toxicity at all. Calne and his associates of Cambridge, England reported the first clinical trials with cyclosporine, and a little more than a year later they published a classical series of observations in recipients of cadaveric kidneys, livers and pancreases (19). For clinical use, Calne et al (19) recommended that cyclosporine be used as the sole immunosuppressive agent. In late 1979, our own trials with cyclosporine were begun, with the conclusion that the optimal use of cyclosporine depended upon its combination with steroid therapy (20).

Our usual practice has been to begin prednisone on the day of operation in a dose for adults of 200 mg on the first postoperative day and with daily decrements of 40 mg/day until 20 mg/day is reached as a maintenance dose in the noncomplicated case after 5 postoperative days. If rejection supervenes in spite of this therapy a second burst of steroid therapy is given. The dose of cyclosporine which we have used has been about 17.5 mg/kg/day.

Less than half of the patients treated in this way have a completely untroubled convalescence. In the rest, adequate renal

function either is not obtained at the outset or else graft deterioration occurs after initially satisfactory function (21). When a secondary decline in renal function occurs, it is necessary to devise changes in therapy that can accommodate either the possibilities of rejection or of cyclosporine nephrotoxicity. The most serious and consistent side effect of the agent has been renal injury, but fortunately this has almost always been responsive to reductions in dose. Our own hypothesis has been that nephrotoxicity and rejection can occur simultaneously (21).

Our initial trials with cyclosporine were in 1979 and 1980. The results were compared with historical controls. In spite of the fact we were engaged in a learning process, the one year actual graft survival after primary cadaveric transplantation was nearly 80% (Table 2).

At the University of Pittsburgh in 1981 a randomized trial was carried out in which the results under cyclosporine-steroid therapy were compared to those with conventional double drug treatment using azathioprine and prednisone. The divergence in results was so great that the trial had to be discontinued within less than a year. The one year primary graft survival was 90% under the experimental protocol compared to than less than 50% using conventional therapy (Table 3). The mortality during 1981 in all groups of patients was 1%.

An important feature of the improved immunosuppression with cyclosporine and steroids has been the ease with which cadaveric

retransplantation has been possible. After retransplantation, our results in the pilot trials at the University of Colorado and subsequently at the University of Pittsburgh have resulted in about a 75% one year cadaveric graft survival (Table 2 and 3), almost double that usually reported and in comparison with the outcome in our own institution for several preceding years. The fact that retransplantation can be so readily carried out with this improved immunosuppression has virtually eliminated any incentive to carry out persistant or excessive attempts at salvaging kidneys undergoing protracted or unusually severe rejection.

FUTURE POLICIES IN TRANSPLANTATION

The conclusions which have reached from observations in the last several years have opened up some areas for lively discussions. Thus what I will speculate upon might be considered to be controversial. My own feeling is that the use of living related donors will become obsolete as a result of the great improvements in immunosuppression and particularly those made possible with cyclosporine-steroid therapy. The role of tissue matching will be diminished in transplantation practices, since it has been so easy to override the immunologic problems caused by mismatches. At the same time it will be increasingly important to have accurate crossmatching techniques since there is no reason to believe that preformed antibody states can be successfully dealt with with cyclosporine-steroid therapy. The importance of sensitization will be an important objective in future times and because of that the preparation of patients by

transfusion which I discussed earlier will become a less and less desirable practice. Diabetics will be easier to treat and the same applies to other patients currently considered to have an increased risk. Thus the criteria for candidacy will be liberalized. It seems certain that the drain of patients from the dialysis centers will become more rapid, but we have been told recently that the numbers entering dialysis will also increase and thus the dialysis pools will not dry up. In any case the interface between dialysis and transplantation will undoubtedly change.

One of the previous speakers has emphasized that the ambience between the transplant surgeons and the nephrologists has sometimes been a hostile one. This will have to change. The nephrologists are going to have to face the fact that transplantation is a reliable service and probably safer than dialysis. Physicians who have withheld patients from cadaveric transplantation because of their dissatisfaction with the results to the present time will be in a position to change their minds. The question which is so paramount in importance here in Kuwait and which exists world wide is the extent to which the organ supply will be a critical limitation in renal transplantation. I think it is vitally important for all nations who wish to serve their own citizens to create a legal structure which will permit and even openly encourage the donation of organs from cadavers and under the appropriate circumstances (including brain death) which will permit a high expectation of success.

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Table 1. Immunosuppressive drug regimens and adjuncts for kidney transplantation

Agent	Year described and reported	Place	Deficiencies
Azathioprine	1962 (4)	Boston	Ineffective, dangerous
Azathioprine-steroids	1963 (3, 22-24)	Denver, Richmond, Boston, Edinborough	Suboptimal
Thoracic duct drainage as adjunct	1963 (17)[a]	Stockholm	Nuisance: requires 20 to 30 days pretreatment
Thymectomy as adjunct	1963 (1)	Denver	Unproven value
Splenectomy as adjunct	1963 (1)	Denver	No longer necessary
ALG as adjunct	1966 (6)	Denver	Suboptimal
Cyclophosphamide substitute for azathioprine	1970 (7)	Denver	No advantage except for patients with azathioprine toxicity
Total lymphoid irradiation	1979 (14, 15)	Palo Alto, Minneapolis	Dangerous; extensive preparation; not quickly reversible
Cyclosporine alone	1978-1979 (19)	Cambridge	Suboptimal
Cyclosporine-steroids	1980 (5, 20, 21)	Denver	Under evaluation

a It was not realized until much later that pretreatment for 3 to 4 weeks before transplantation was a necessary condition (16).

Table 2. Cadaveric graft and patient survival in first cyclosporine trial, from December 1979 to September 1980

	At 6 mo	At 12 mo	At 18 mo	At 25 to At 24 mo	36 mo
First grafts (57 in 57 patients)	48 (84.2%)	45 (79%)	44 (77.2%)	43 (75.4%)	39 (68.4%)
Retransplants (10 in 9 patients)	6 (60%)	6 (60%)	6 (60%)	6 (60%)	6 (60%)
Survival of the 66 patients	58 (87.9%)	57 (86.4%)	57 (86.4%)	56 (84.8%)	52 (78.8%)

Table 3. Cadaveric graft and patient survival in second cyclosporine (and control) trial (1981)

	At 3 mo	At 6 mo	At 9 mo	At 11 mo	At 11 to 21 mo
Primary grafts with cyclosporine-steroids (N=38)	36 (94.7%)	35 (92.1%)	35 (92.1%)	35 (92.1%)	35 (92.1%)
Primary grafts with azathioprine-steroids (N=32)	22 (68.6%)	17 (53.1%)	16 (50%)	15 (46.9%)	14 (43.8%)
Retransplants with cyclosporine-steroids (N=29 patients)	24 (80%)	23 (76.6%)	23 (76.6%)	23 (76.6%)	20 (66.7%) [b]
Survival in all 99 patients[a]	98 98	98	97	96 (97%)	

a Two deaths after 2 weeks and 18 months were with functioning grafts (one each in the cyclosporine and retransplantation series) and were caused by myocardial infarction and ruptured abdominal aneurysm. The third patient (azathioprine series) was anephnic and died of gastrointestinal hemorrhage 9 ½ months after transplantation.

b Two of the 3 late graft losses were from chronic rejection after 12 and 13 months; the third was from death (ruptured aneurysm) after 18 months.

Figure 1. Classic rejection crisis in patient treated 20 years ago. The donor was a sibling. Deterioration of renal function began more than 2 weeks after transplantation. All stigmata of rejection were present except for acute hypertension and weight gain, which were successfully prevented by medical treatment. Acti-C- Actinomycin C; LN - Left nephrectomy at time of transplantation; RN - Right nephrectomy. Imuran is synonymous with azathioprine. (By permission of Surg Gynec Obstet (117:385, 1963.

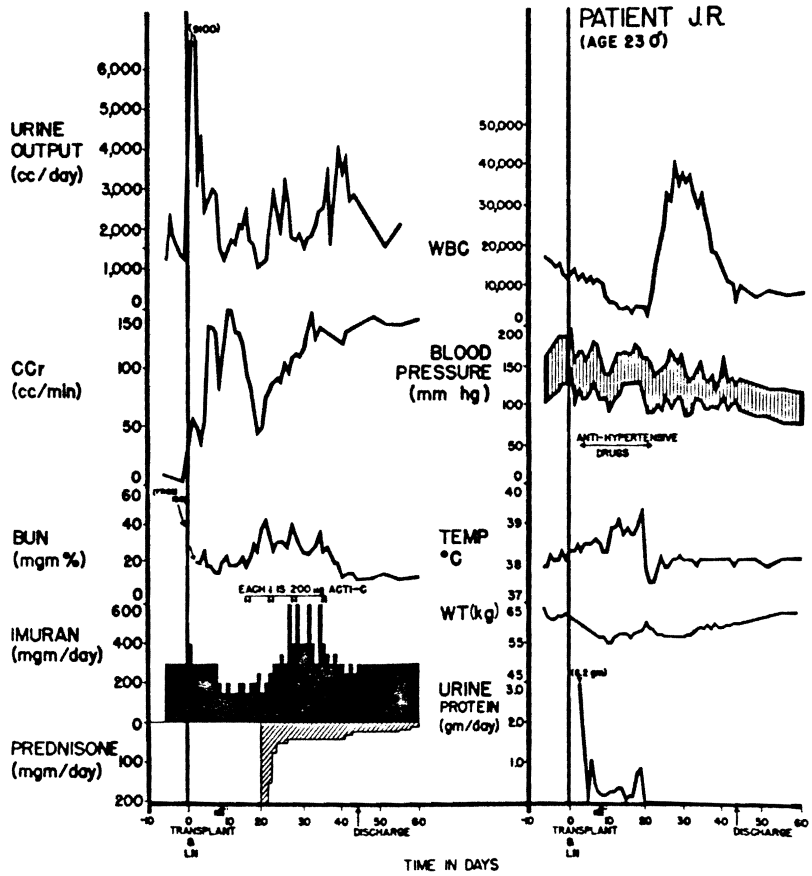
PATIENT J.R.
(AGE 23 ♂)
URINE OUTPUT (cc/day)
CCr (cc/min)
BUN (mgm%)
IMURAN (mgm/day)
PREDNISONE (mgm/day)
WBC
BLOOD PRESSURE (mm hg)
TEMP °C
WT(kg)
URINE PROTEIN (gm/day)
ANTI-HYPERTENSIVE DRUGS
TRANSPLANT & I.R.
DISCHARGE
TIME IN DAYS

FIGURE 1