The future of organ transplantation: from the laboratory to the clinic

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This is a short review of tolerance from the point of view of the clinician. Various examples of tolerance occurring in patients and animal models that relate to the clinical experience are described. It is suggested that there may be different mechanisms by which tolerance is achieved, but from the patient's point of view operational tolerance is the goal, whereby, after a short induction procedure, the patient will maintain good function in the grafted organ indefinitely without maintenance immunosuppression. It is pointed out that such a goal may be difficult to achieve with any given protocol due to the enormous variation between donors and recipients of organ grafts of tissue matching, innate immune reactivity and susceptibility to disturbance of a tolerant state by infections or allergic reactions. Thus the case is made for *prope* or almost tolerance in which graft acceptance is maintained by a low, non-toxic dosage of maintenance immunosuppression that may not be required indefinitely.

Keywords: tolerance; organ transplantation

1. INTRODUCTION

Up until the end of the 1950s the possibility of transplanting organs to restore to normal life patients afflicted with fatal diseases was a dream, which had a mythological basis in the legend of Saints Cosmos and Damien. The Saints replaced a cancerous leg with a leg from a recently buried Moor. The operation was a success and the idea was so attractive to the medieval church that there are beautiful illustrations of the surgical procedure throughout Europe showing Saints Cosmos and Damien, who, themselves, were identical twins, performing this miraculous operation.

At the end of the fifth decade of the last century the possibility that the dream might become a reality was raised by two separate developments. The first was the demonstration that vascularized organs could be grafted and experimental autotransplantation of the kidney was shown to be repeatable with a high percentage of success. In Boston, USA, Murray and his colleagues transplanted kidneys between identical twins and showed that a kidney transplant would restore to health a uraemic patient (Murray *et al.* 1955). It also raised the ethical question of removal of a normal organ from a healthy person, a beginning of disquieting problems of a moral nature that have become increasingly important in clinical transplantation.

The other advance, spearheaded by Medawar and his colleagues, was the demonstration that graft rejection had an immune basis (Gibson & Medawar 1943). The body reacted against a foreign graft in a similar manner to invading bacteria and viruses. The immune reaction was effected by the reticuloendothelial system, particularly lymphocytes, and having once experienced an individual foreign graft, the body was primed for an extremely rapid despatch of a second graft from the same source, hence the term 'immune reaction'.

Billingham, Brent and Medawar also showed a way of preventing graft rejection by challenging the developing immune system in the embryo or neonate with the putative antigen in question (Billingham *et al.* 1953). They first found that this occurred naturally in non-identical cattle twins, which share their blood circulation in the placenta. They were then able to repeat this effect in planned experiments, joining eggs together in parabiosis and injecting cells from the donor mice of one inbred strain into the potential foetal or neonatal recipient of another inbred strain. The chickens, offspring of the parabiotic eggs, accepted skin grafts from the parabiont and the foetal or neonatally injected mice were similarly tolerant to the donor of the cell injection.

Unfortunately, for obvious reasons, experimental immunological tolerance could not be used in the clinic and alternative methods of preventing rejection were sought. The first approach was to destroy the whole of the reticuloendothelial system, including the bone marrow, and replace it with bone marrow cells from the potential donor. Evolution of this strategy has been very successful in bone marrow transplants but the relationship between donor and recipient needs to be close, usually a sibling of identical tissue type (Thomas *et al.* 1959). Such patients will accept kidney grafts from the bone marrow donor without any further immunosuppression but the initial ablative immunosuppression requires powerful and toxic irradiation and/or drugs (Sayegh *et al.* 1991; Helg *et al.* 1994; Jacobsen *et al.* 1994).

The second approach, which became applicable for organ transplantation, was to use anti-leukaemic drugs to interfere with graft rejection, particularly azathioprine, a chemical derivative of 6-mercaptopurine (Calne 1961). This, together with fairly high doses of corticosteroid drugs, provided the first successful protocol for clinical kidney grafting (Murray *et al.* 1963).

Concurrently, a complex system of tissue types was discovered, dependent on the expression of genes from the sixth human chromosome. Matching was found to be of importance in the prognosis of organ grafting, particularly between related donors. In addition, respect for the ABO red blood cell groups was also necessary, fulfilling the same requirements as with blood transfusion.

Numerous compounds were investigated as immunosuppressants, for the most part without success. Anti-lymphocyte antibodies produced in the serum of animals injected with human lymphocytes were sometimes powerful inhibitors of the immune system, although for many years batch characteristics were variable and unpredictable. Improvements have occurred in the production of anti-lymphocyte serum and a whole new area has developed with monoclonal antibodies, each of which has a single molecular target (Kohler & Millstein 1975).

It was nearly 20 years later before cyclosporin A (Neoral), a new and very effective immunosuppressant, became available. Although nephrotoxic, Neoral, when judiciously combined with the other known agents, transformed the practice of transplantation so that it became a general form of treatment instead of an experimental endeavour in a very few centres (Calne *et al.* 1978). Following the introduction of Neoral, transplantation of the heart, liver and later lungs and pancreas became established.

There are a number of new immunosuppressants that have entered the clinic and are, or soon will be, registered. The results of organ transplantation worldwide have improved so that, now a victim of its own success, transplantation cannot be offered to more than a small percentage of patients who might benefit. There is an increasing disparity in the number of organs available and those patients requiring treatment. Despite much effort and expense, organ donation from cadavers has not increased in the last decade and consequently there has been more attention devoted to persuading relatives, spouses and friends to give organs, sometimes subjecting them to considerable pressure without the doctor knowing. The cost of maintenance immunosuppression in terms of financial resource is high and is a barrier to organ transplantation in many developing countries. Patients receiving continuous immunosuppression are susceptible to infections, malignancy and also the side-effects of each of the immunosuppressive drugs. Although the early results of organ transplantation are excellent, by ten years half the grafts will be lost mainly due to slow, irreversible chronic rejection and the side-effects of the drugs (Nagano & Tilney 1997; Cecka 1998).

2. STATE OF THE ART

Recipients of unrelated cadaveric and poorly matched grafts have lived up to 30 years with still-functioning transplants, so it is possible to get excellent survival and many of the patients have been able to resume work and outdoor activities, including vigorous exercise. Unfortunately, many patients do not aspire to such good long-term results. There is a danger of diminishing returns by just adding more and more powerful drugs that interfere with the immune system. Immune reactions switch off

naturally, but high dose maintenance immunosuppression may interfere with this process.

Pigs and rats with vascularized liver allografts can become tolerant without any drug treatment. Acute rejection occurs but resolves spontaneously (Calne *et al.* 1969). Some liver allografted patients can stop maintenance immunosuppression without penalty. Minute doses of maintenance immunosuppression may be all that is necessary for long-term good function in renal allografts. Cessation can lead to prompt rejection (Woodruff *et al.* 1976; Campos *et al.* 1984). A short course of Neoral accompanied by an infusion of donor bone-marrow-derived cells can result in tolerance to experiment renal allografts in pigs (Calne *et al.* 1994) and myeloablative regimens followed by donor bone marrow infusions can lead to tolerance of renal allografts in primates (Thomas *et al.* 1999).

Recent experimental data would support cautious optimism that tolerance or *prope* (almost) tolerance (see below) protocols should soon be successful in the clinic. The profound T-cell depletion resulting from CD3-diphtheria immunotoxin treatment of rhesus monkeys by Knechtle *et al.* (1997) led to renal and subsequent skin graft tolerance in a majority of animals. The mechanism has not been clarified, but establishment of a renal graft in a milieu devoid of T cells may swing the balance of immune regulation towards graft acceptance. The kidney transplants were performed seven days after the first dose of immunotoxin (Knechtle *et al.* 1997). Grafting at the time of immunotoxin dosage did not produce tolerance. Thomas and colleagues have data supporting a hypothesis that cytokine activation by the immunotoxin produces a vascular leak syndrome that interferes with tolerance induction, and they have shown that a short course of 15-deoxyspergualin together with methylprednisolone prevents the cytokine activation and that stable tolerance ensued in a majority of animals (Contreras *et al.* 1998). Concordant islet xenografts survived and functioned, reversing spontaneous diabetes in monkeys treated with immunotoxin and five days of Neoral, a remarkable achievement (Thomas *et al.* 1999). Non-depleting interference with co-stimulatory pathways has led to prolonged renal allograft survival in rhesus monkeys. These data are discussed elsewhere in this issue (and see also Kirk *et al.* 1997).

Although tolerance would be the ideal goal, the heterogeneity of donor recipient combinations, immune status, underlying disease and unpredictable consequences of infection might make stable tolerance extremely difficult to achieve for all patients. The prospect for settling for a short pulse of antibody, followed by monotherapy, may be a reasonable compromise, ensuring that no patient is put at risk of rejection should graft acceptance be broken by intercurrent infection, or other inciting events. We have coined the term '*prope* (almost) tolerance' to offer some respectability to this concept.

We have used a powerful lymphocyte-depleting antibody, CAMPATH-1H, with half-dose Neoral monotherapy maintenance immunosuppression in 31 recipients of cadaveric renal allografts.

CAMPATH-1H, a humanized anti-CD52 antibody, is a powerful lytic agent for both T and B lymphocytes but does not affect bone marrow stem cells (Hale *et al.* 1988).

Table 1. Summary of CAMPATH-1H renal recipients

(Abbreviations: Tx, transplant; PCK, polycystic kidney disease; GN, glomerulonephritis; A, Alport's disease; DM, diabetes mellitus; HT, hypertension; I, idiopathic; IgA, IgA nephropathy; FSGN, focal sclerosing glomerulonephritis; IN, interstitial nephritis; P, pyelonephritis; UTI, urinary tract infection; CyA, cyclosporin A (Neoral); Aza, azathioprine; Pred, prednisolone; IHD, ischaemic heart disease; TB, tuberculosis; CMV, cytomegalovirus; I/S, immunosuppression; Tac, Tacrolimus. Reproduced from Calne *et al.* (1999) by permission of *Transplantation*.)

patient	age	sex	Tx date	diagnosis	current creatinine $\mu\text{mol l}^{-1}$	current lymphocytes $\times 10^9 \text{l}^{-1}$	rejection	events
1	55	F	18/06/97	PCK	122	1.1		
2	65	F	08/08/97	PCK	110	0.9	yes	rejection day 60: steroid responsive: ?UTI
3	31	M	15/08/97	DM	108	0.8		
4	30	F	05/09/97	DM	117	1.2		
5 ^a	51	M	02/10/97	PCK	124	0.5		
6 ^a	42	M	03/10/97	I	128	0.9		
7 ^b	34	M	27/10/97	GN	325	1.0	yes	rejection day 35 ischaemia/UTI CyA, Aza, Pred
8	36	F	27/10/97	PCK	78	2.2		
9	44	F	29/10/97	HT	134	1.3		
10	36	F	01/11/97	GN	467	2.4		recurrent IgA nephropathy
11	42	M	05/11/97	I	208	1.7		
12	68	M	05/11/97	GN	131	1.6		
13 ^c	60	M	10/11/97	PCK	400	1.2		dead IHD 10-98
14	36	F	25/04/98	IgA	103	0.7		
15	48	M	26/04/98	GN	124	0.4		
16	22	M	26/04/98	GN	122	0.3		intra-abdominal TB
17	50	M	28/04/98	IgA	223	2.3	yes	rejection day 28: Pred added: CMV
18	63	F	28/04/98	GN	222	0.5		
19	24	F	04/05/98	FSGN	113	1.0		
20	40	M	05/05/98	IgA	142	1.4		
21	36	M	05/05/98	A	133	1.1		
22	28	M	13/05/98	GN	141	0.4		
23 ^a	35	M	24/05/98	HT	330	0.9		
24	59	F	20/06/98	GN	120	0.7	yes	rejection day 30 change of I/S: Tac and Pred
25	34	F	01/07/98	P	106	0.6		
26	35	M	01/07/98	GN	154	1.0	yes	rejection day 50
27	56	F	02/07/98	PCK	114	0.9		
28	40	F	06/07/98	IN	72	1.0		
29	27	F	07/07/98	IgA	177	1.8		
30	21	M	12/07/98	GN	147	0.6		
31	65	F	25/07/98	PCK	139	1.0		

^a Asystolic donor.

^b Thrombosis of inferior of three arteries with ureteric and renal infection.

^c Severe congestive cardiac failure prior to transplantation.

The same antibody had in the past been able to reverse rejection episodes (Friend *et al.* 1955) and had therefore demonstrated efficacy in a study that provided a basis for calculation of antibody dosing. We used this antibody in low doses given to recipients of cadaveric kidney allografts.

Immunosuppressants may overwhelm the patient's immune system and prevent the development of natural active graft acceptance. Long-term immunosuppression carries the dangers of infection, cancer, renal damage, hypertension, diabetes, hyperlipidaemia, hirsutism, Cushingoid facial appearance and bone necrosis. If maintenance immunosuppression is stopped, rejection usually follows except in some liver transplant patients.

Strategies to establish tolerance will probably require the initial use of immunosuppressive agents, as an induct-

ion treatment to prepare the immune system for active graft acceptance and then very-low-dose maintenance immunosuppression to guard against rejection.

Our intent was to reduce T- and B-cell counts by 1 or 2 logs by infusion of the lympholytic antibody CAMPATH-1H peritransplant. Lymphocyte depletion was followed by half-dose monotherapy with Neoral instituted on the third post-operative day. No other immunosuppressive drug was given.

We reported on the first 13 patients in 1998 (Calne *et al.* 1998). We have now transplanted a total of 31 consecutive patients over the period of study of 18–30 months (Calne *et al.* 1999).

Thirty-one patients with kidney failure, not previously transplanted, received cadaveric renal allografts (two

were from asystolic donors) and two doses of 20 mg of intravenous CAMPATH-1H. Five of the patients were given the first dose just before surgery and the second dose 24 hours later. The remaining 26 were given the first dose shortly after surgery and the second 24 hours later. In the absence of an aggressive T-cell response, a presumed engagement of certain donor and recipient donor-marrow-derived cells may be beneficial, and might even be necessary to establish tolerance. A window of opportunity for immunological engagement (WOFIE) may be needed for this to take place (Calne 1997). Therefore a gap for all patients of 48 hours was left without further immunosuppression. Neoral was then started at a dose aimed to produce maintenance trough concentrations of 75–125 ng ml⁻¹. Methylprednisolone (500 mg) was given intravenously 30 minutes before the first dose of CAMPATH-1H to minimize reactions to cytokine release. Lymphocyte cytotoxicity tests between recipient serum and donor cells were negative in all cases. HLA mismatches varied from one to five. Standard renal allografting surgical techniques were used. Patients were given routine antibiotic prophylaxis and acyclovir if there was a cytomegalovirus recipient–donor mismatch. Cotrimoxazole was given for six months and amphotericin B lozenges for six weeks.

Twenty-nine patients have sustained function in their grafts between four and 20 months after surgery. Twenty-six are receiving low-dose Neoral as monotherapy. Three patients (cases 7, 17 and 24 in table 1) are on prednisolone (10 mg d⁻¹) and 100 mg d⁻¹ azathioprine after rejection episodes.

All recipients had immediate and profound depletion of circulating lymphocytes. Counts remained very low for two to six months and then recovered quite quickly (figure 1). Five patients had episodes of impaired renal function. In case 2 the biopsy was not typical of rejection but methylprednisolone was given along with antimicrobials for *Klebsiella* urinary infection. In cases 7, 24 and 26 there was acute cellular rejection but in case 17 the appearances were an almost acellular vasculitic rejection. One patient required a second pulse of steroid and the immunosuppression was changed to conventional triple therapy. There were no serious side-effects attributable to the monoclonal antibody. Two infections occurred. One a cytomegalovirus (CMV) systemic infection that responded to a course of intravenous ganciclovir, and the other an abdominal abscess requiring a right hemicolectomy probably due to a reactivation of an old tuberculous infection. The patient had been born and brought up in Thailand. This infection responded to anti-tuberculous treatment.

We are currently studying the *in vitro* reactivity of the repopulated lymphocytes in the renal recipients in an attempt to determine how 'tolerant' these graft recipients have become after having received CAMPATH-1H. We are also monitoring the antibody titres in sensitized patients. A randomized trial is planned to compare the protocol described in this report with conventional treatment.

Although the follow-up in this series of patients is relatively short, there has been no evidence to date of progressive worsening of renal function in any of the patients that might be a marker of chronic rejection. The

Figure 1. CAMPATH-1H and mean creatinine after transplant. Reproduced from Calne *et al.* (1999) by permission of *Transplantation*.

patients with raised serum creatinine levels are tending to fall except in one patient, number 10, who has recurrence of her original disease with slowly deteriorating function. The protocol initially adopted has not been interfered with, however, and there would be innumerable drug combinations that could be tried; but, in view of the results obtained, we felt it was best to adopt the protocol described above for the forthcoming randomized trial.

We need in future studies to determine the efficacy of this protocol in sensitized patients, to determine whether any immunological penalty is incurred long term, and to optimize the dosing parameters for induction and maintenance therapy.

However, if the excellent progress shown by most of our patients continues, then this protocol for induction and maintenance immunosuppression has a number of potential advantages when compared with current practice. First, it is simple and cheap, with special implications for countries with tight budgetary constraints; and second, corticosteroids, with all their attendant side-effects, have been avoided in most cases and the regimen is less susceptible to non-compliance.

A strict mechanistic definition of tolerance is unhelpful, since an 'operationally tolerant state', all that is needed for the welfare of a patient, may be achieved by different mechanisms. A useful working definition would be the long-term functional graft survival in a patient not requiring maintenance immunosuppression. Thus, by analogy tolerance could be compared with the concept of happiness, something that can be achieved in different ways. On occasion we do not know when we are blessed with happiness, but we are usually acutely aware of the loss of happiness. So tolerance, again like happiness, may be complete or partial and it can be lost by a low grade, ill-understood, chronic rejection or the precipitation of acute rejection by another stimulus, for example, viral infection or an allergic response. A minimal baseline, non-toxic dose of maintenance immunosuppression could possibly guard against these hazards, but then the state of the patient would be almost (or *prope*) tolerance. Some patients might not require the maintenance immunosuppression, but at present we cannot determine this by any means except withdrawal of the maintenance drug.

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